Regulatory Networks (4)

model building

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- General consideration
- Examples

  gene expression
  TCR signaling
  p53
  NF-κB

What is model building ?

We have

some (not enough) chemical rules/reaction rates

some observations of the system dynamics

We want to construct a model, which follows chemical rules and system dynamics, is solvable and has some predictive power.

Inverse problem to solve:
we know the system dynamics,
we do not know the dynamical sytem

Regulatory Motifs

Feedbacks: negative (homeostasis, stochasticity control)
positive (bistability)

Time delays: stiff – transcription (~ 40bp/s)
– translation (15 a.a.)

: distributed – transport
– modifications
– intermediates

Negative feedback + time delay → oscillations (supercritical Hopf)
Positive feedback + negative → oscillations (subcritical Hopf, SNIC bifurcation), bistability (*yes* or *no* signaling)

kinetic proofreading

Regulatory Motifs

Kinase cascades → signal amplification

Non linear elements:

modifications (phosphorylation, ubiquitination etc.)
dimerization (polimerization)
scafolds
and many others (NF- κB -- IκBα)

Transient activity (IKK kinase)

Stochasticity in regulatory networks

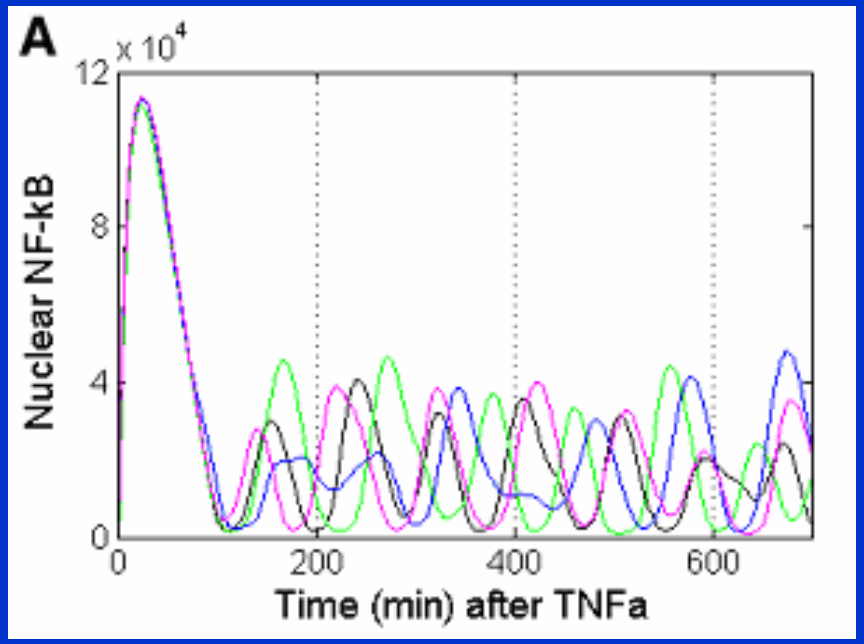
Stochastic system ? Consider its deterministic limit.

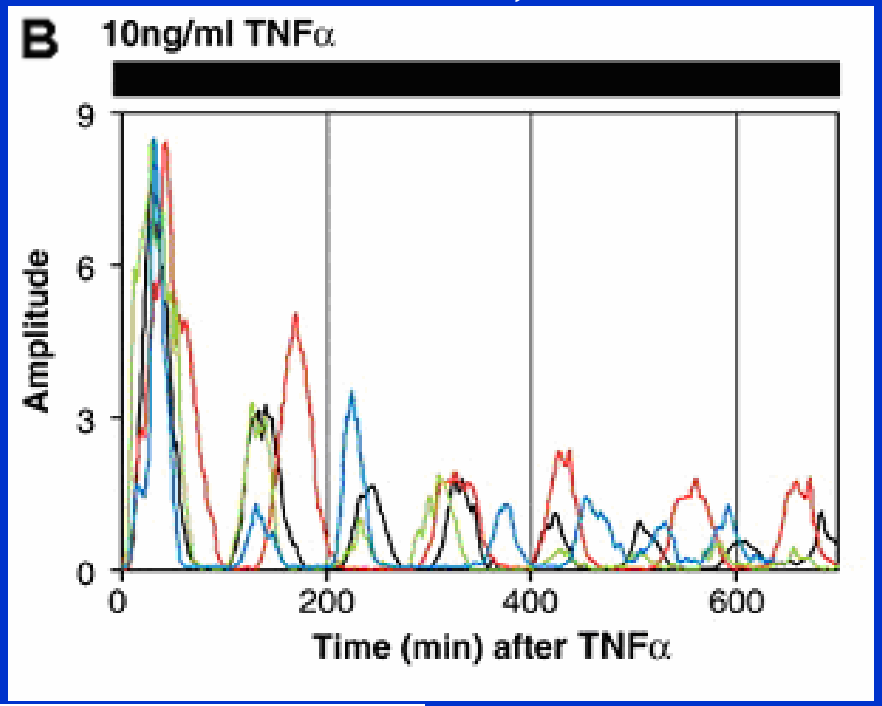
- Stochasticity is not as important when the system has only one stable steady state

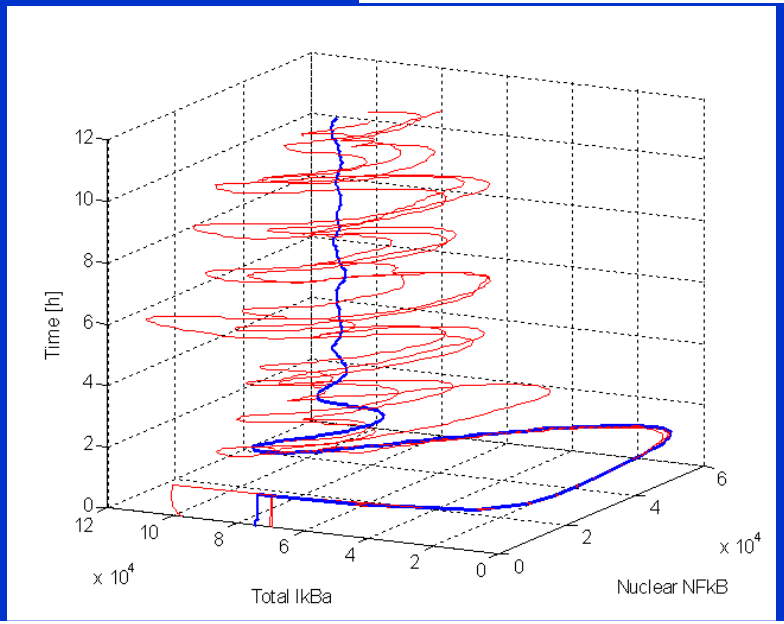
- Stochasticity is important for data interpretation and model building when system has stable limit cycle

- Stochasticity is important for cell dynamics and fate when the system has two or more stable steady states or limit cycle

Model predictions and single cell data Nelson et al, Science 2004.



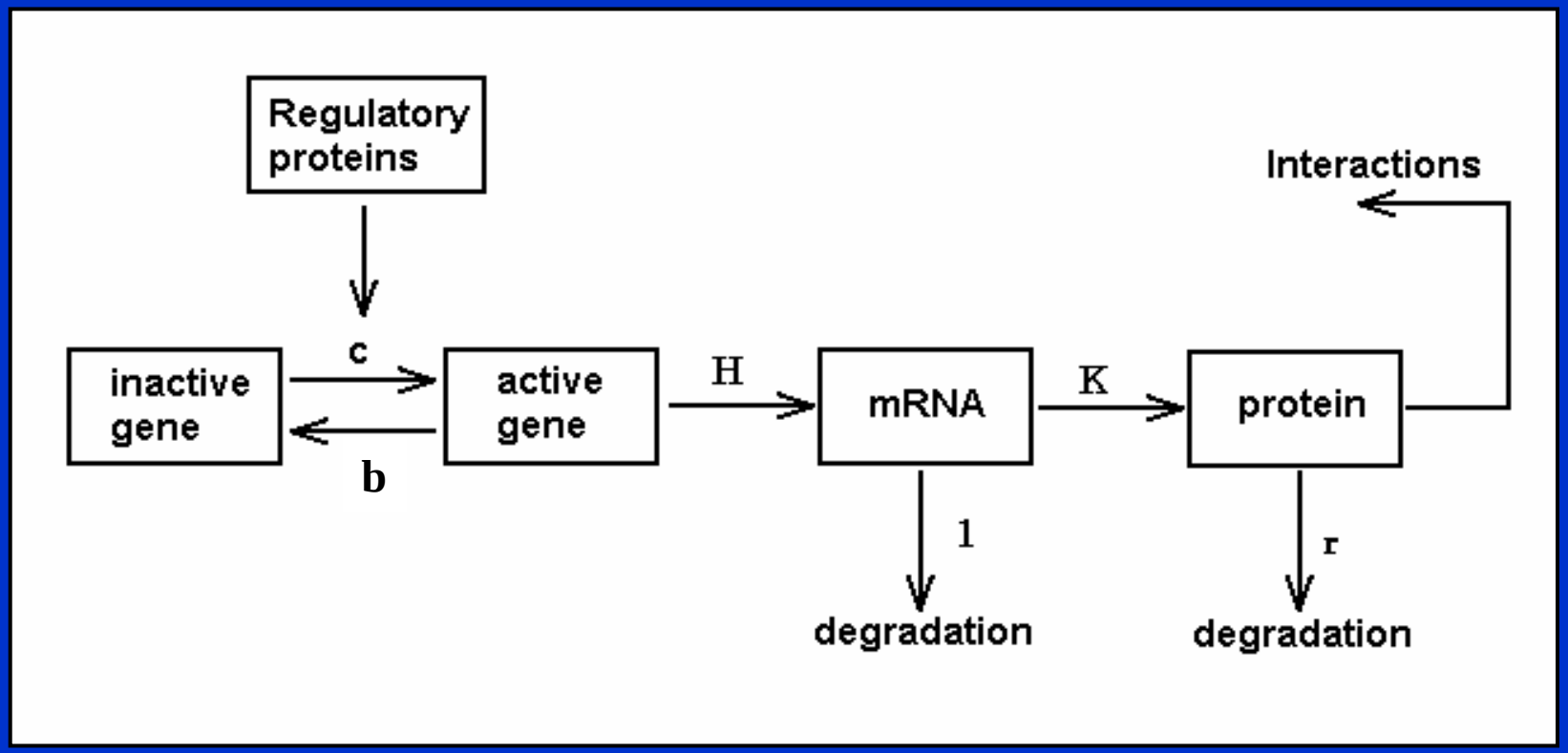




Bistability in gene expression

(Over)simplified schematic of gene expression

- Regulatory proteins change gene status.



- The number of molecules involved:

$$1 \le DNA \le mRNA \le protein \le 10^6$$

Protein is directly produced from the gene

Stochastic

$$\frac{dy(t)}{dt} = -y(t) + G(t)$$

$$G(I) = 0, \quad G(A) = 1$$

$$I \xrightarrow{c(y)} A, \quad A \xrightarrow{b(y)} I,$$

Deterministic

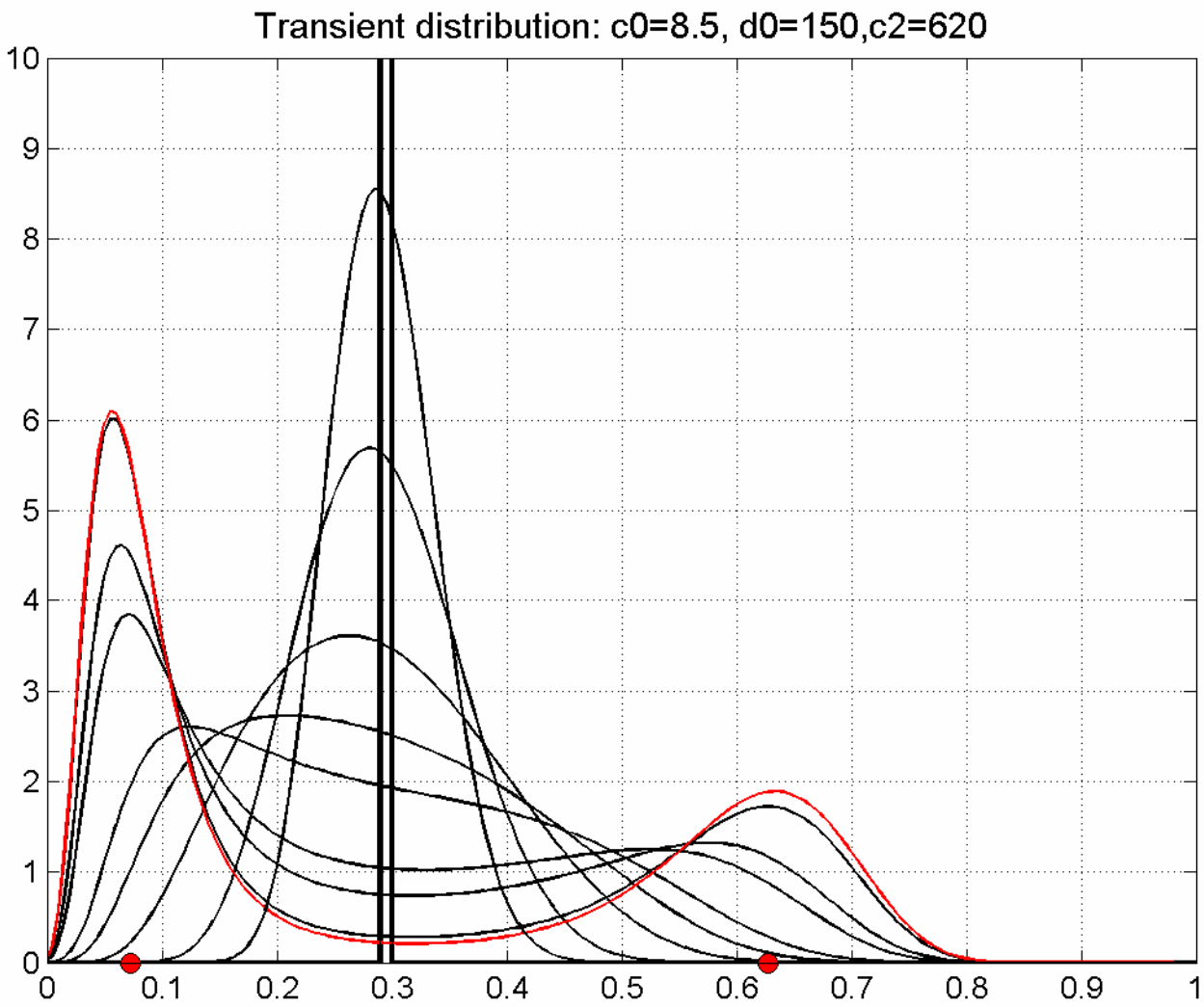
$$\frac{dy(t)}{dt} = -y(t) + E(G),$$

$$E[G] = \frac{c(y)}{c(y) + b(y)},$$

For $c(y) = c_0 + c_1 y + c_2 y^2,$ $b(y) = b_0 + b_1 y + b_2 y^2$

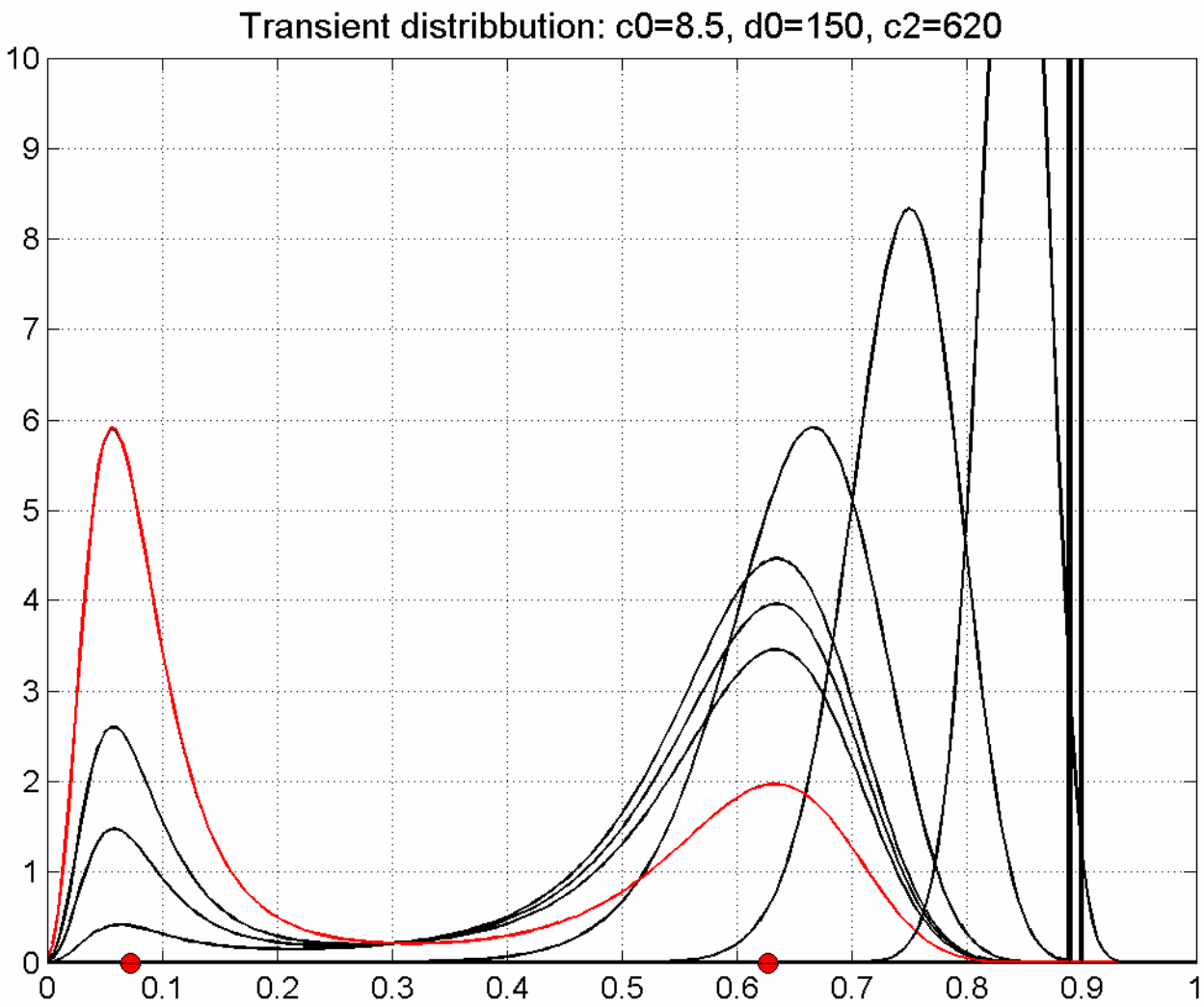
Deterministic system has one or two stable equilibrium points depending on the parameters b_i , c_i

Transient probability density functions



Stable deterministic solutions are at 0.07 and 0.63

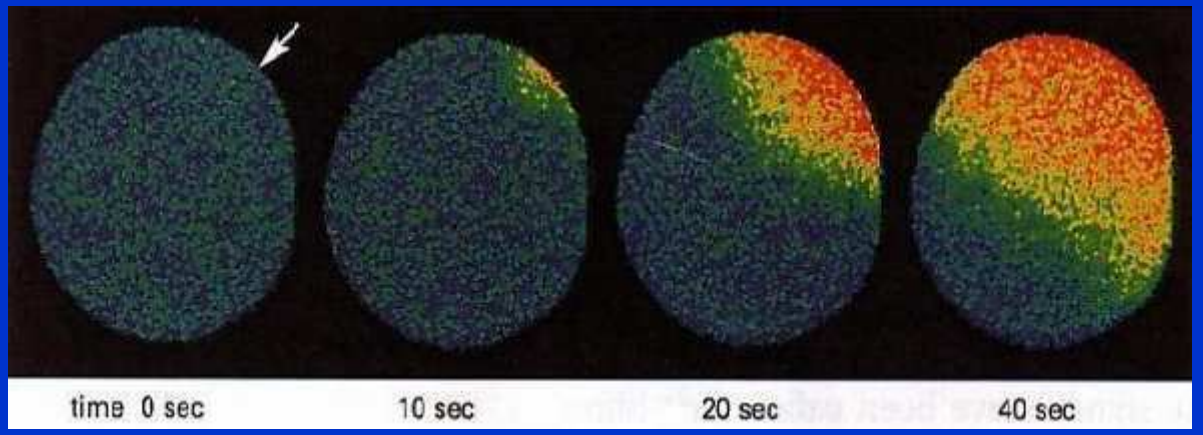
Transient probability density functions



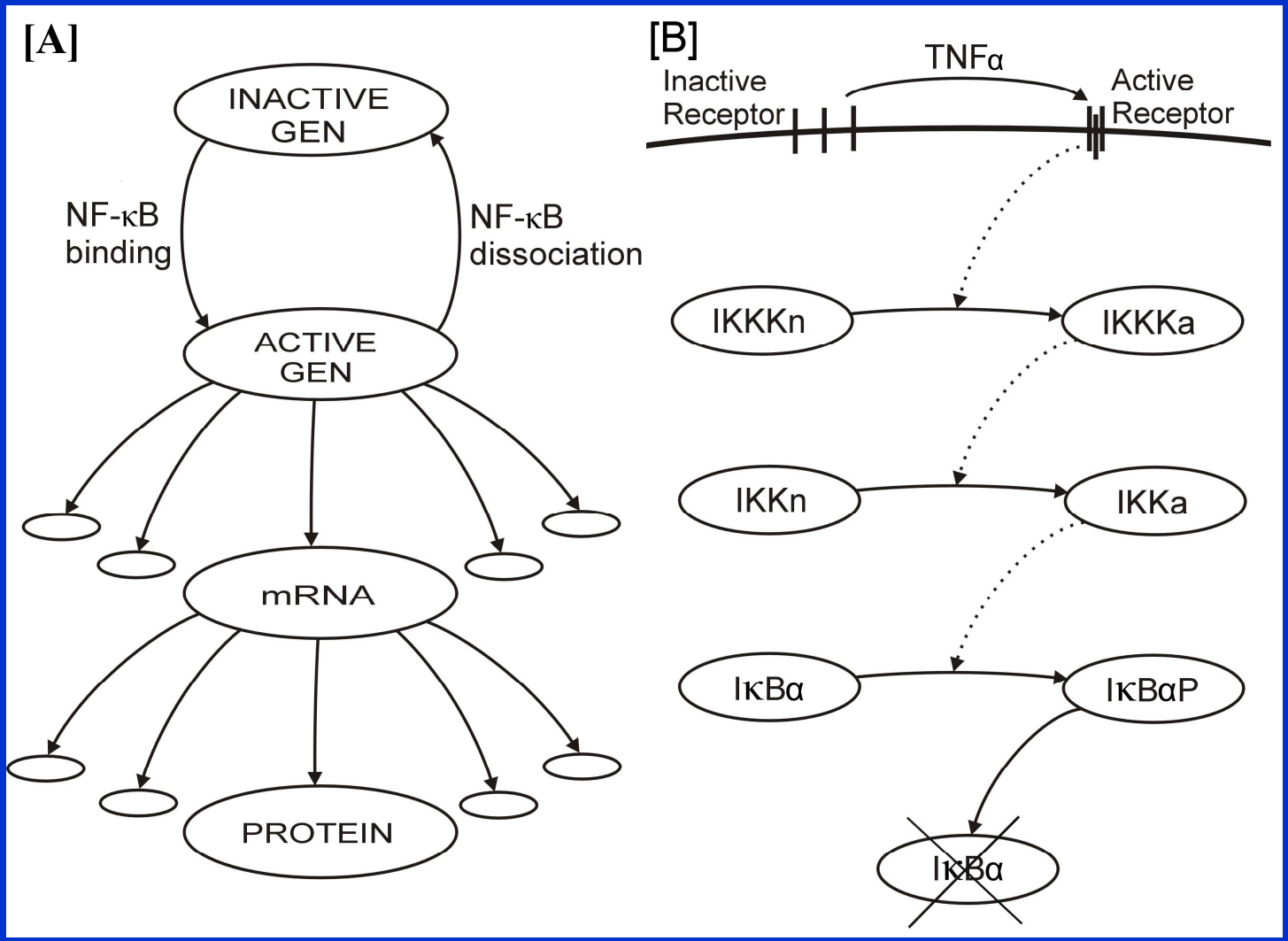
Stable deterministic solutions are at 0.07 and 0.63

Stochastic switches and amplification processes

- gene activation → transcription →translation
- receptor activation → kinase cascade (TCR, TNFR)
- Calcium fluxes (calcium channels are open by calcium)



Stochastic switches and amplification cascades



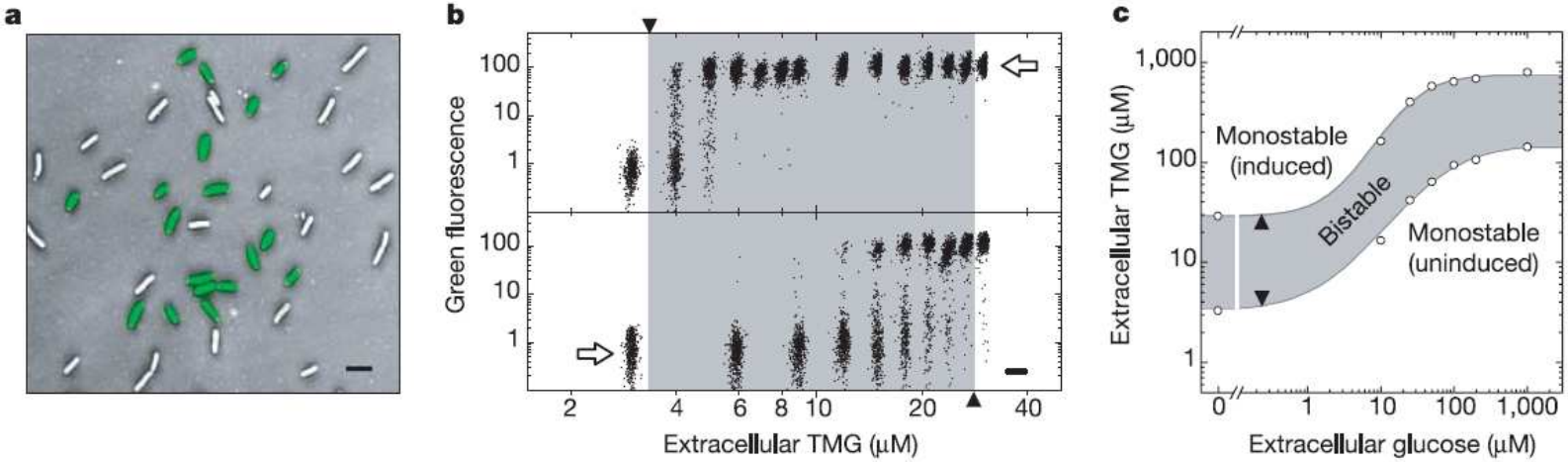


Figure 2 Hysteresis and bistability in single cells. **a**, Overlayed green fluorescence and inverted phase-contrast images of cells that are initially uninduced for *lac* expression, then grown for 20 h in 18 μM TMG. The cell population shows a bimodal distribution of *lac* expression levels, with induced cells having over one hundred times the green fluorescence of uninduced cells. Scale bar, 2 μm. **b**, Behaviour of a series of cell populations, each initially uninduced (lower panel) or fully induced (upper panel) for *lac* expression, then grown in media containing various amounts of TMG. Scatter plots show log(green fluorescence) versus log(red fluorescence) for about 1,000 cells in each population. Each scatter plot is centred at a position that indicates the underlying TMG concentration. The scale bar represents variation in red fluorescence by a factor of 10. White arrows indicate the initial states of the cell populations in each panel. The TMG concentration must increase above 30 μM to turn on initially uninduced cells (up arrow), whereas it must decrease below 3 μM to turn off initially induced cells (down arrow). The grey region shows the range of TMG concentrations over which the system is hysteretic. **c**, The phase diagram of the wild-type lactose utilization network. When glucose is added to the medium, the hysteretic region moves to higher levels of TMG. At each glucose level, the lower (down arrow) and upper (up arrow) switching thresholds show those concentrations of TMG at which less than 5% of the cells are in their initial states.

Ozbuzdak et al, Nature 2004.

Bistability and stochasticity in T cell receptor signaling

Tomasz Lipniacki – PAS, Warsaw, Poland

Beata Hat – PAS, Warsaw, Poland

William Hlavacek – Los Alamos

James Faeder – Pittsburgh U Medical School

T-cells = T lymphocytes

T-cells govern the adaptive immune response in vertebrates.
T-cells are activated by foreign antigens (peptides).

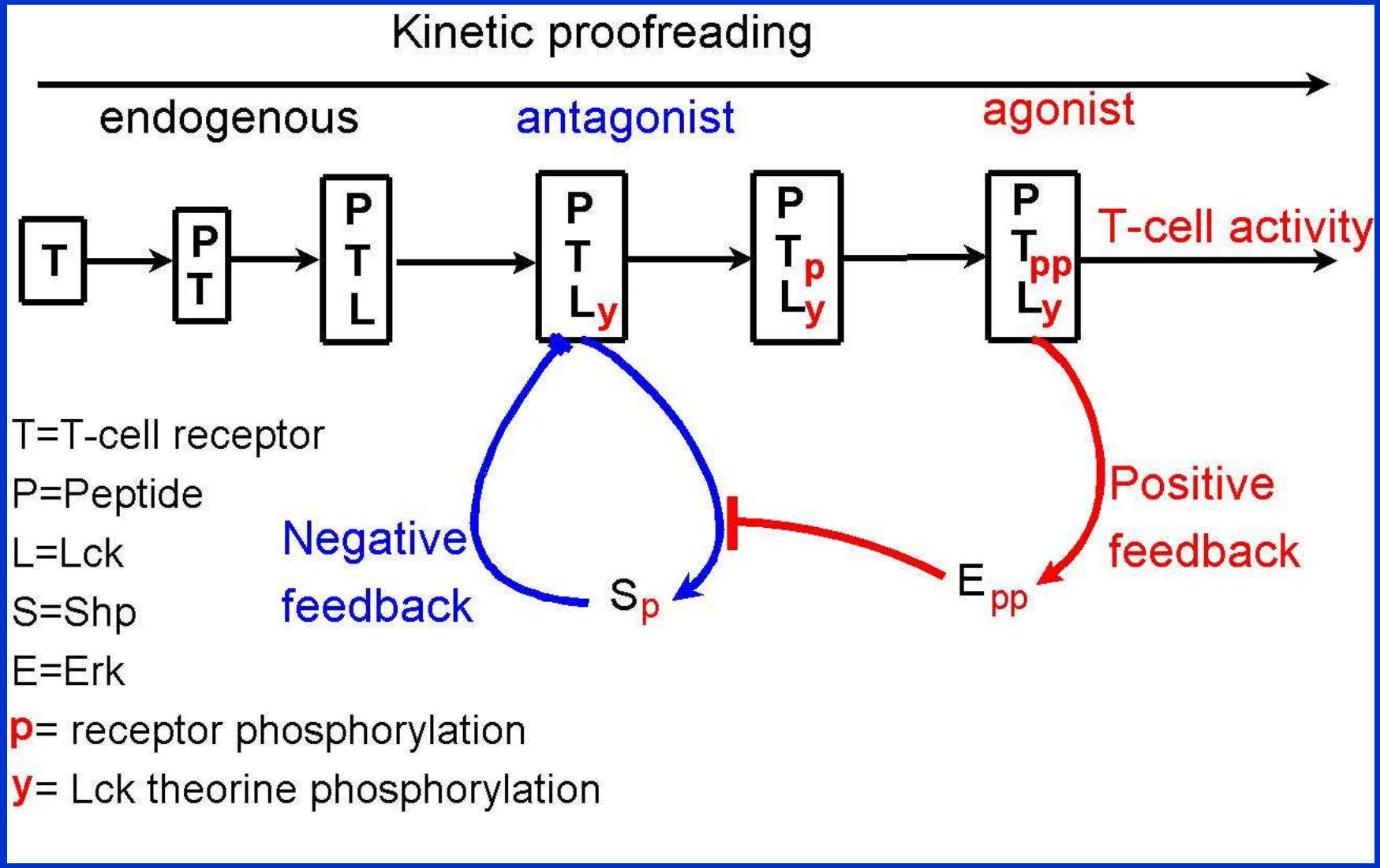
Two main types of T-cells: helper and cytotoxic.

Helper T-cells: when activated secrete cytokines inducing B-cells to proliferate and mature into antibody secreting cells.

Cytotoxic (killer) T-cells: when activated induce apoptosis in cells on which they recognize foreign peptides. They act on fast scale of order of few minutes.

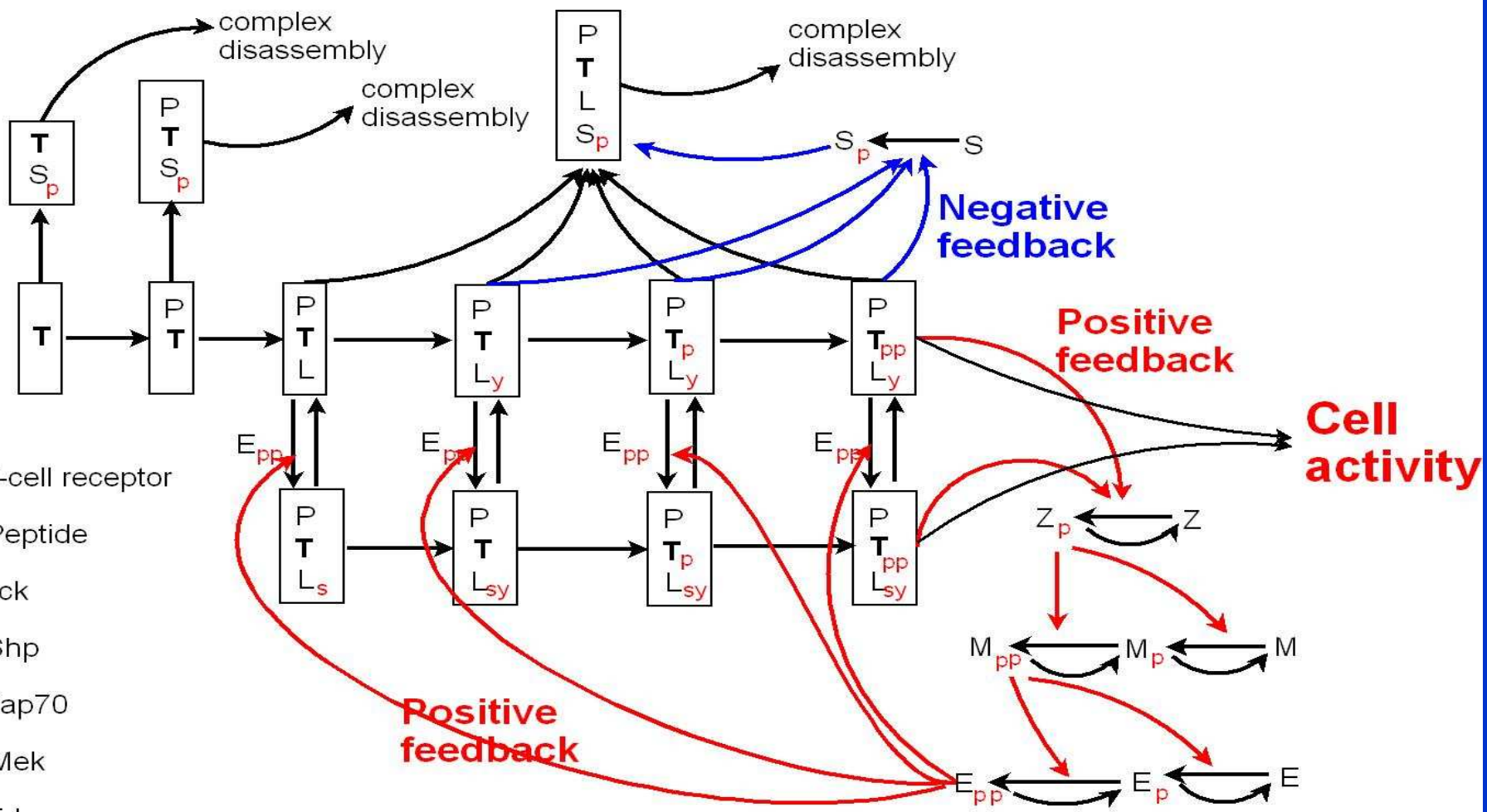
Facts

- High number (100 000) of endogenous peptides with binding time of ~ 0.01-0.1 second have no effect on cell activity.
- Few agonist peptides/cell with binding time > 10s → high activity
- Peptides with binding time of ~ 1s are antagonistic – they do not stimulate T-cells, and also inhibit T-cell activation resulting from stimulation by agonist peptides.

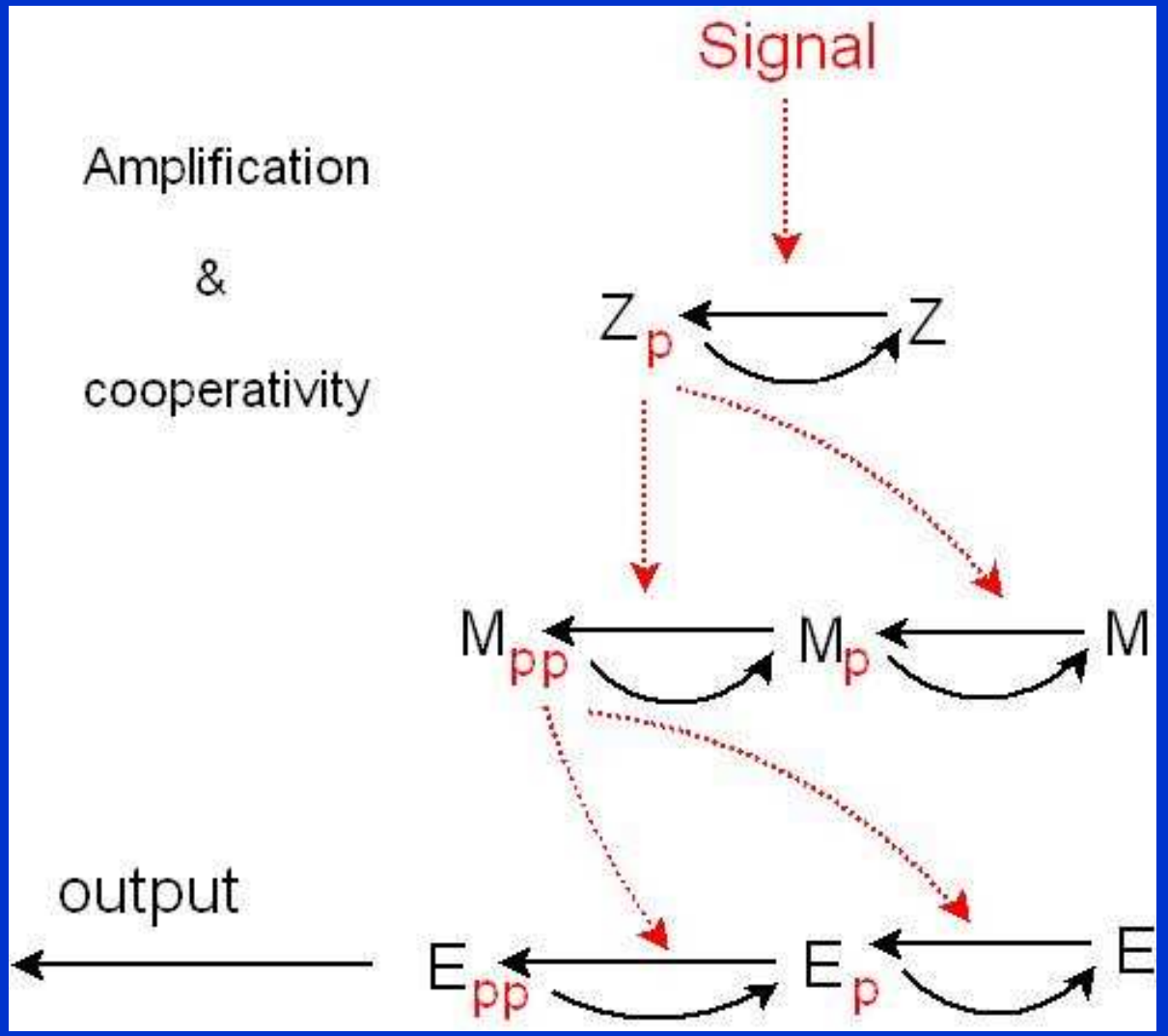


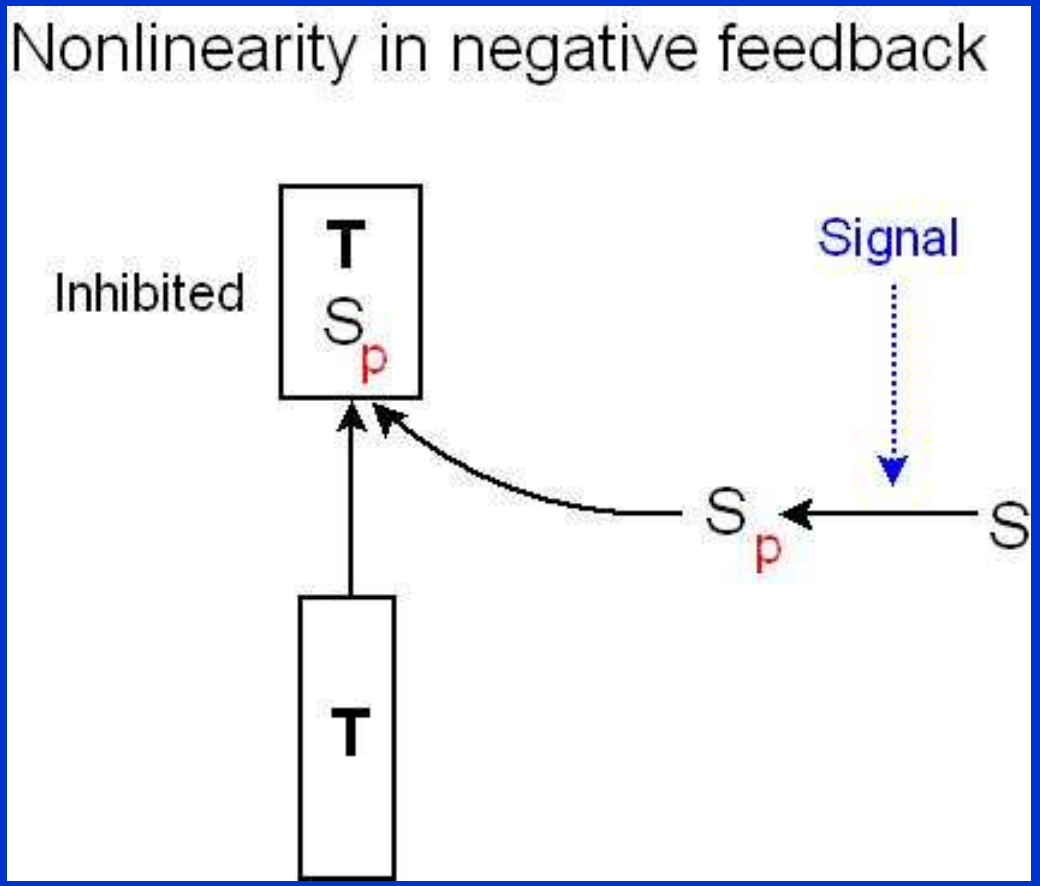
Rabinowitz 1996, Stefanova 2003, Altan-Bonnet 2005

Model of T-cell receptor signalling



Peptide (P) dissociation results in immediate complex disassembly and Lck (L) and TCR (T) dephosphorylation


Signal
Amplification
&
cooperativity
Z
Zp
M
Mp
Mpp
E
Ep
Epp
output

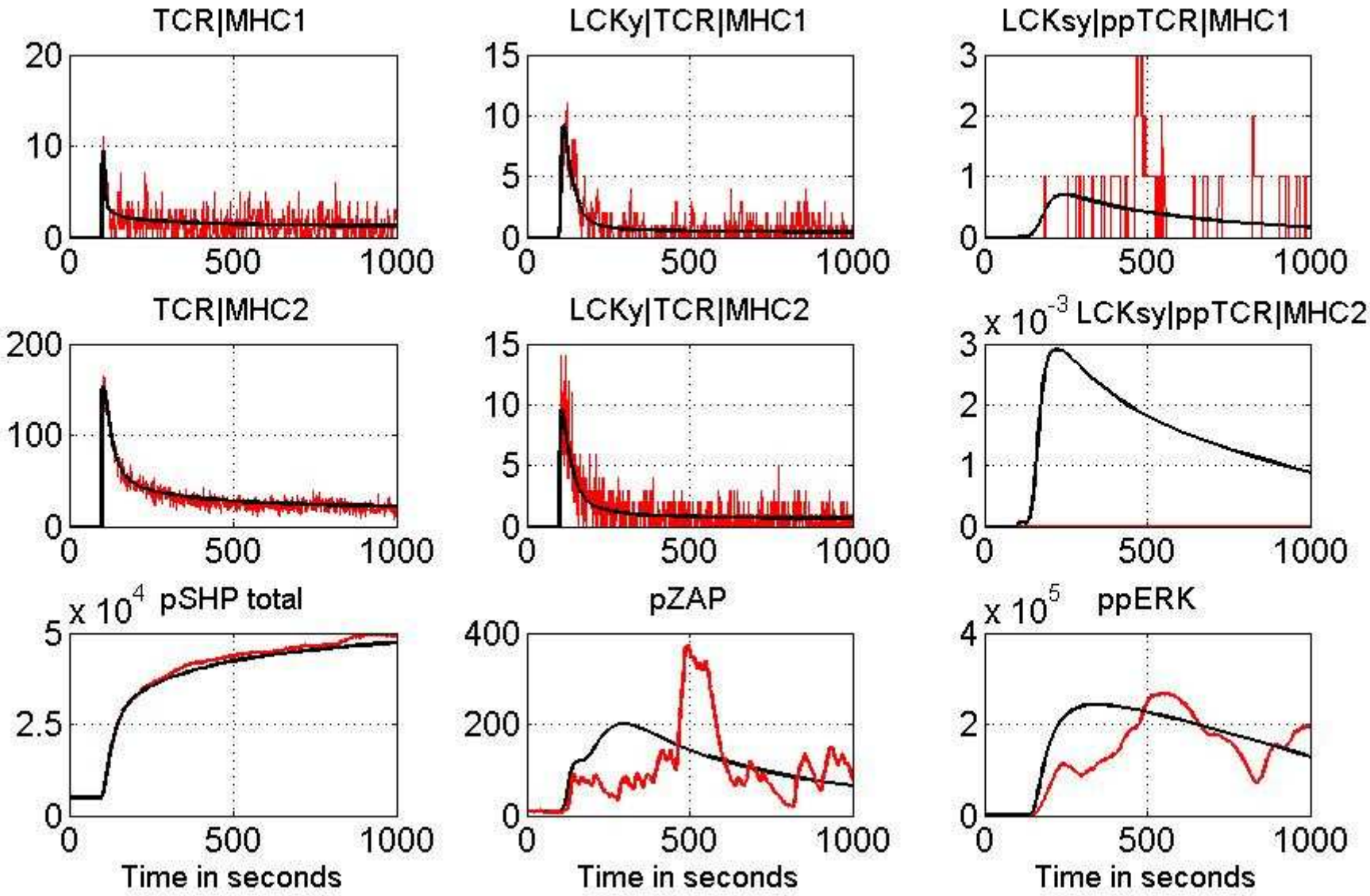

Nonlinearity in negative feedback
Inhibited
T
S_p
Signal
S_p
S
T

Mathematical representation

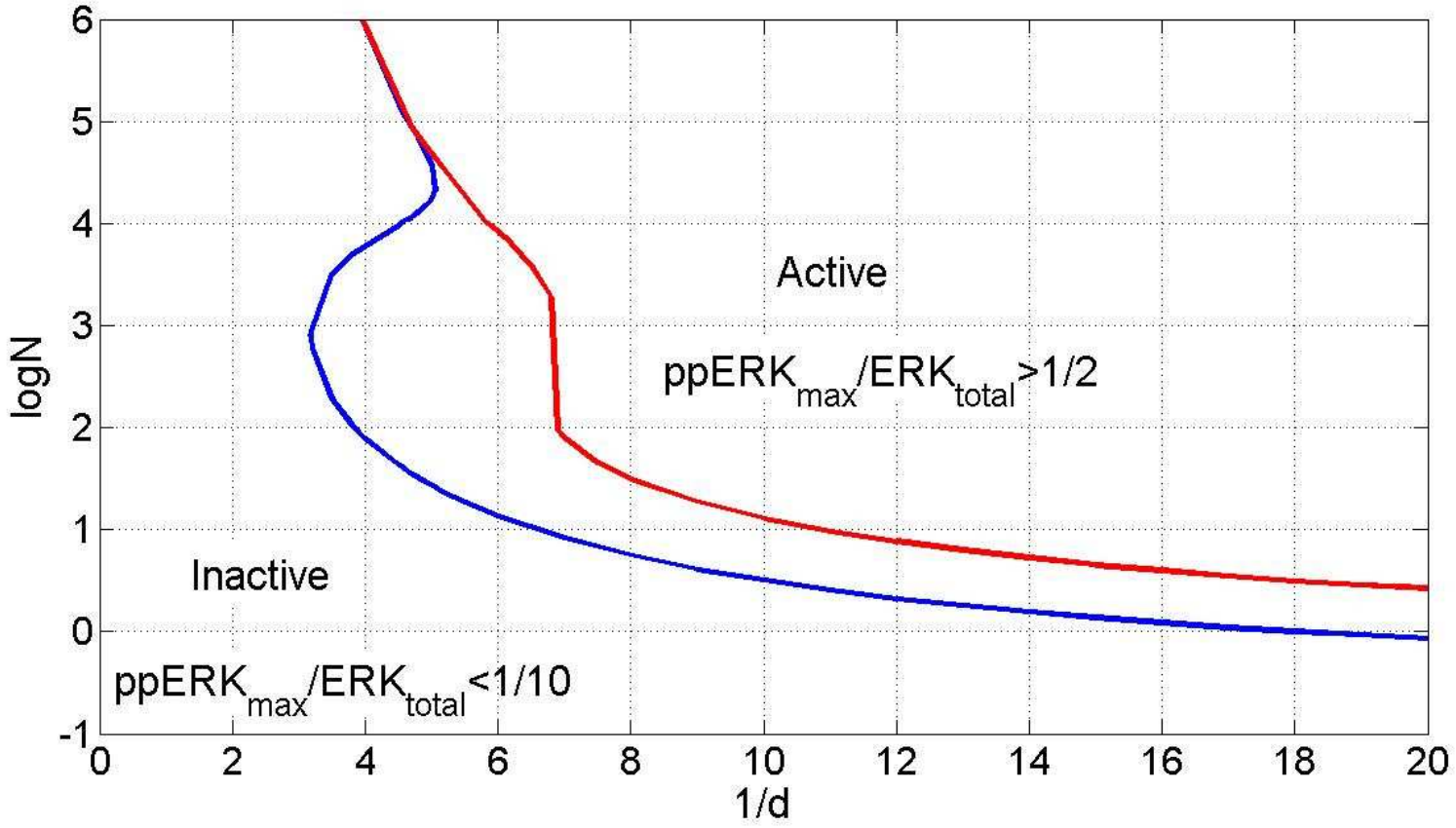
1. Deterministic: 37 ordinary differential equations with 97 chemical reactions.

2. Stochastic: 97 reactions simulated using direct stochastic simulation algorithm, Gillespie 1977.

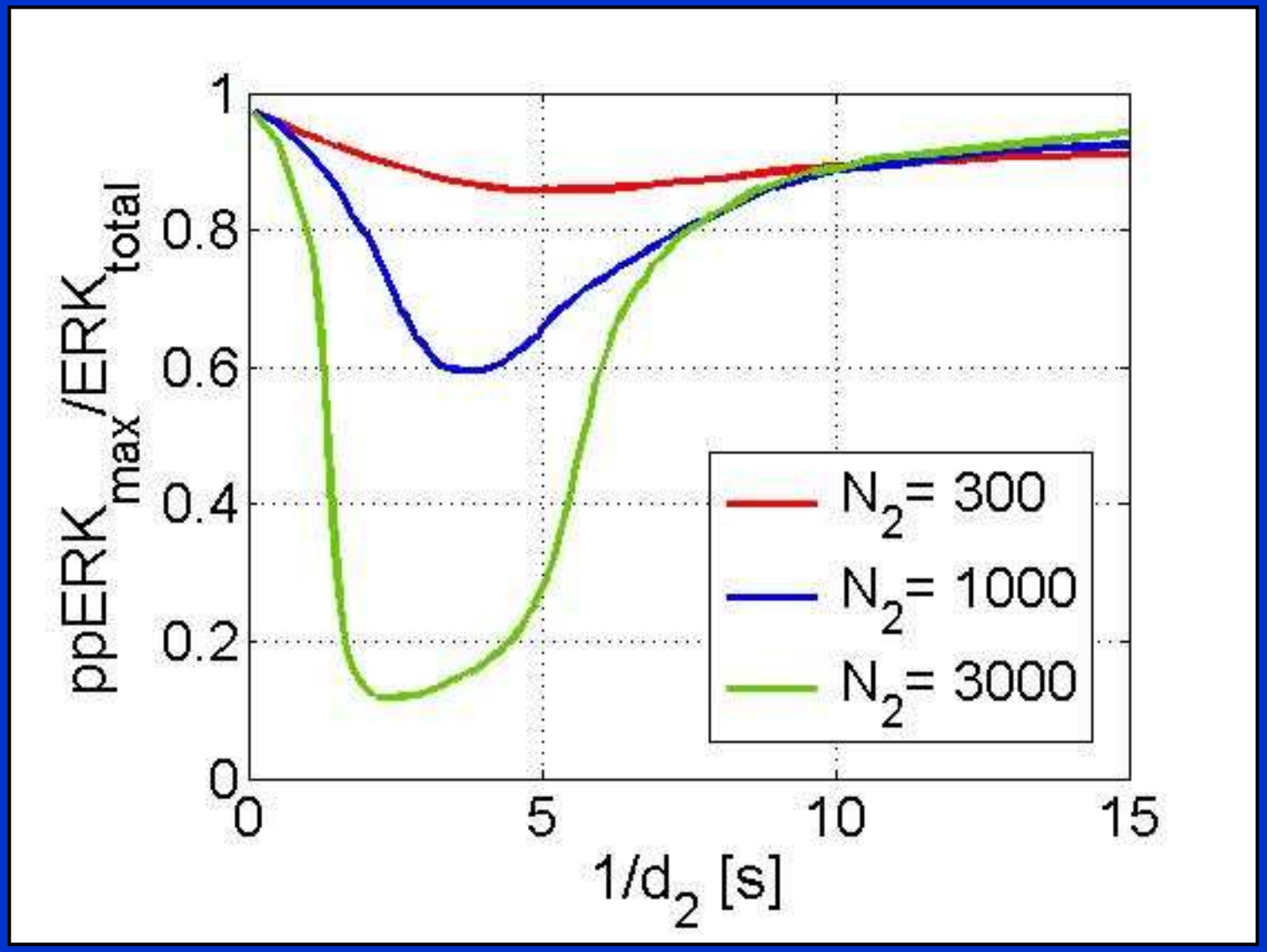
Use BioNetGen ! It goes 100 time faster than Matlab


TCR|MHC1
LCKy|TCR|MHC1
LCKsy|ppTCR|MHC1
TCR|MHC2
LCKy|TCR|MHC2
x 10^-3 LCKsy|ppTCR|MHC2
x 10^4 pSHP total
pZAP
x 10^5 ppERK
Time in seconds
Time in seconds
Time in seconds

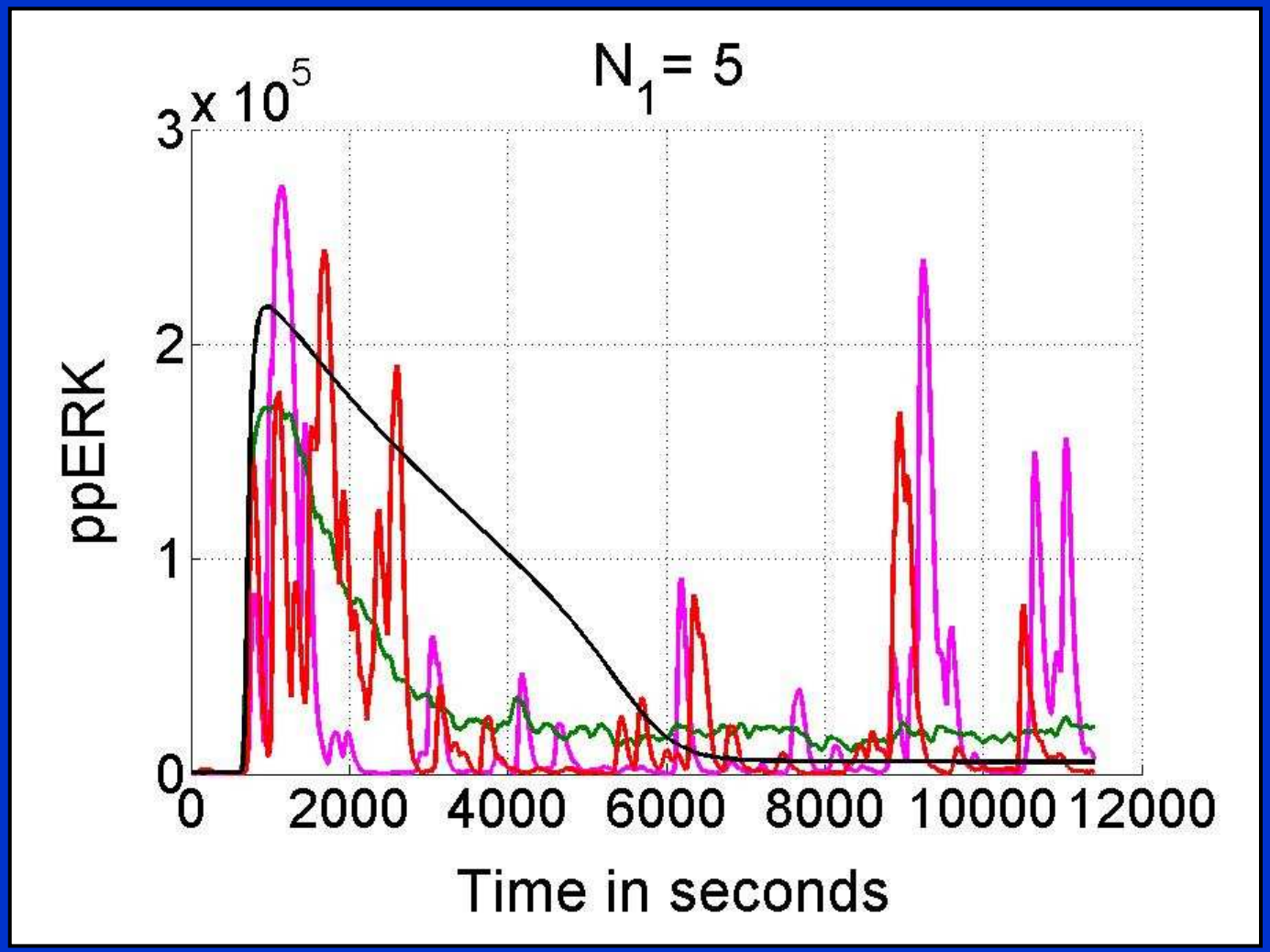
Kinetic discrimination

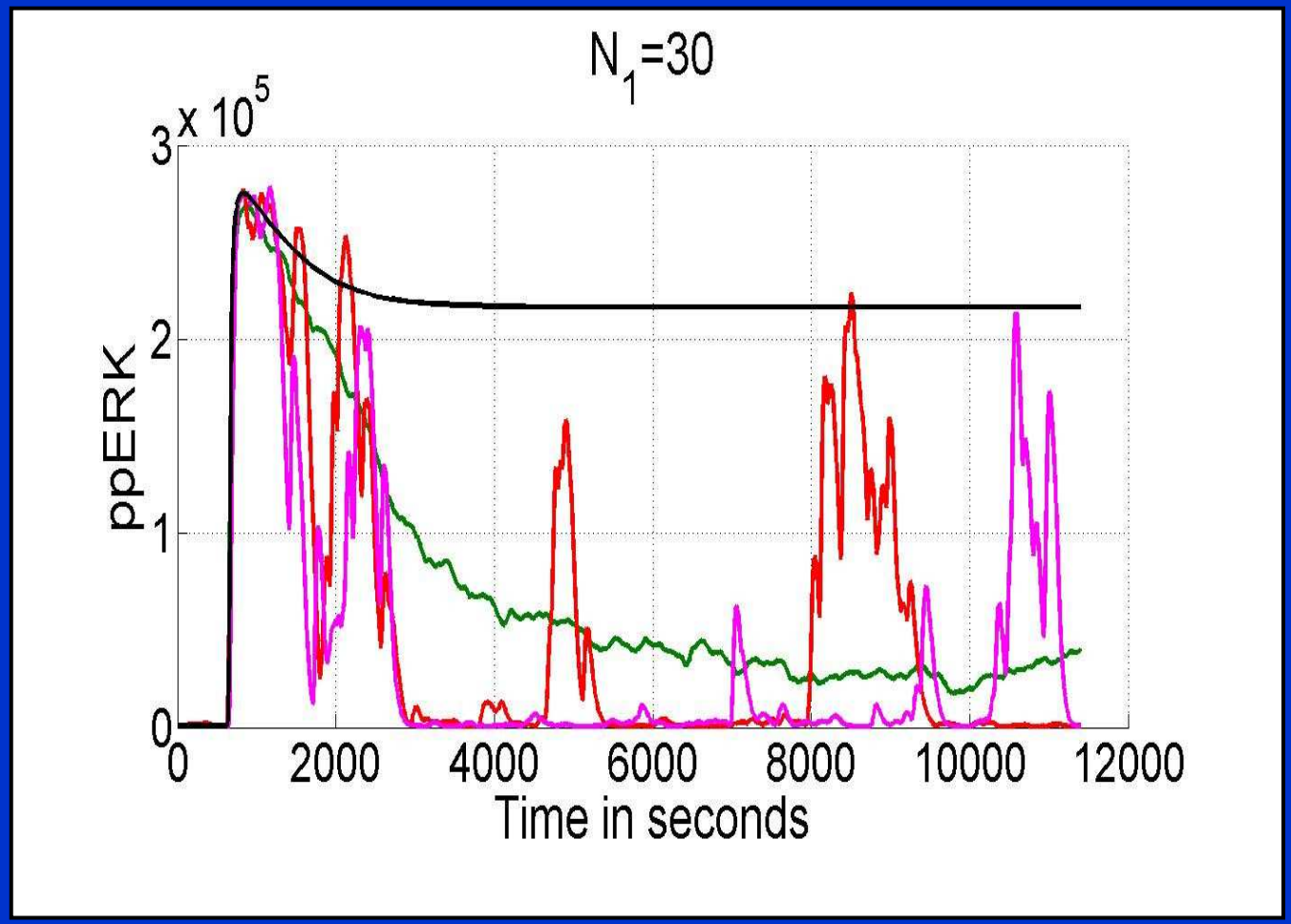


Antagonisms

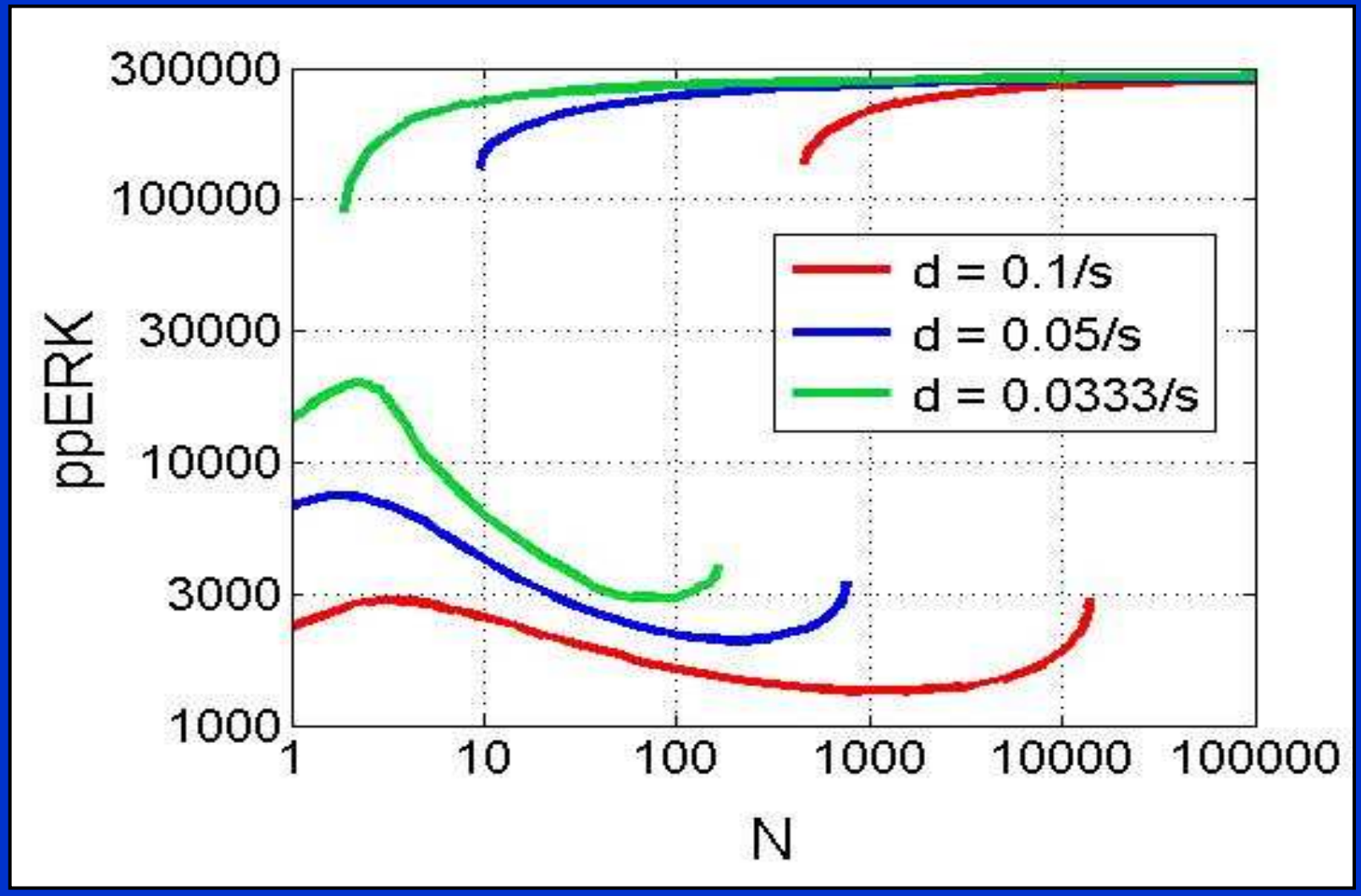


Stochastic versus deterministic trajectories

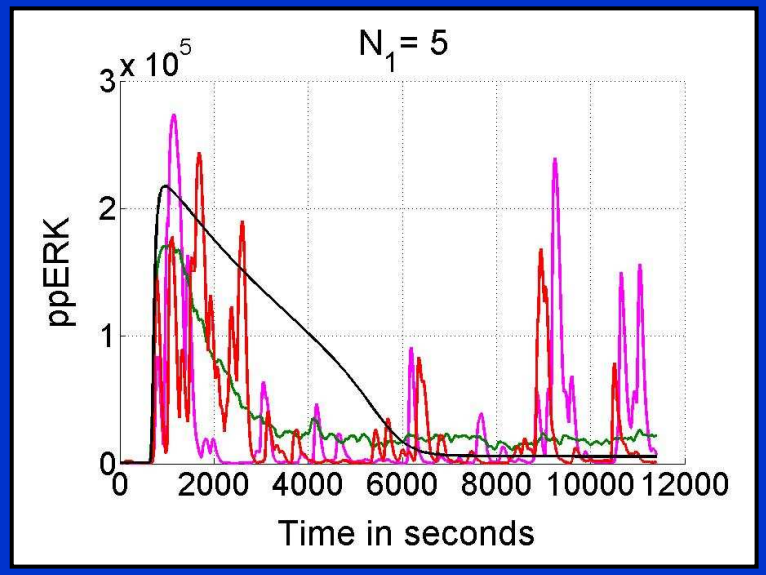


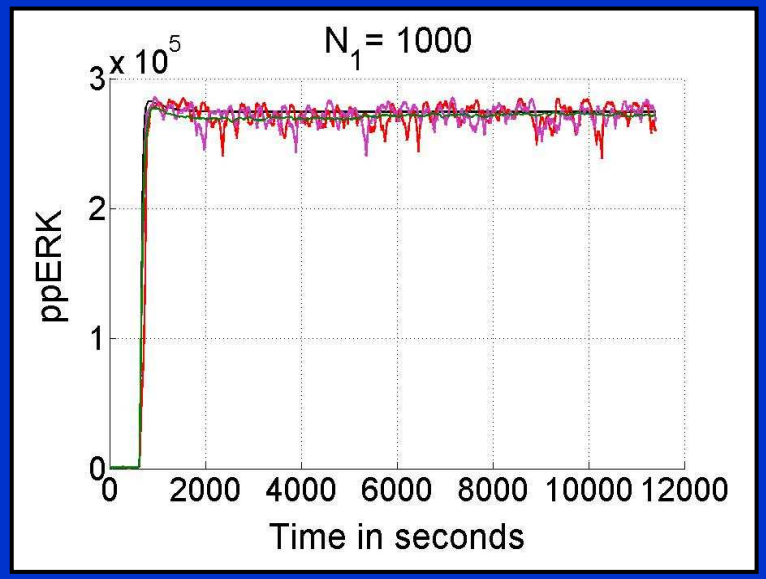

N1=30
x 10^5
3
2
1
0
ppERK
0
2000
4000
6000
8000
10000
12000
Time in seconds

Bistability

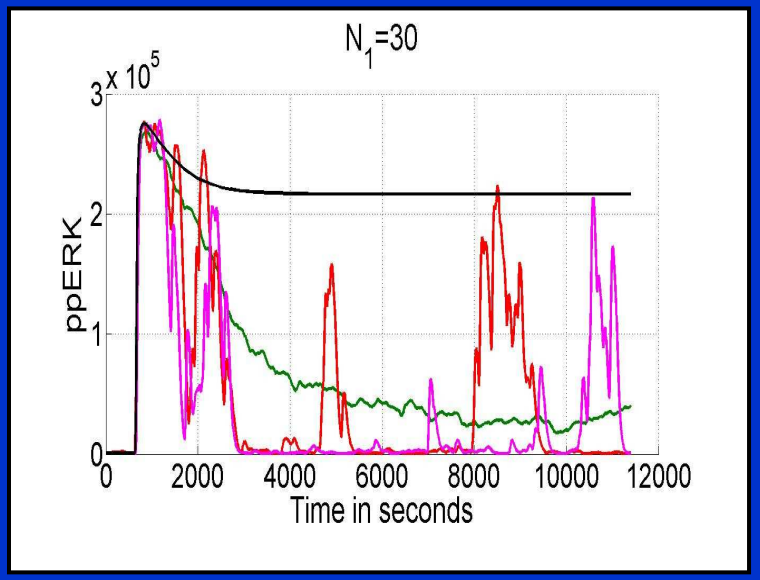


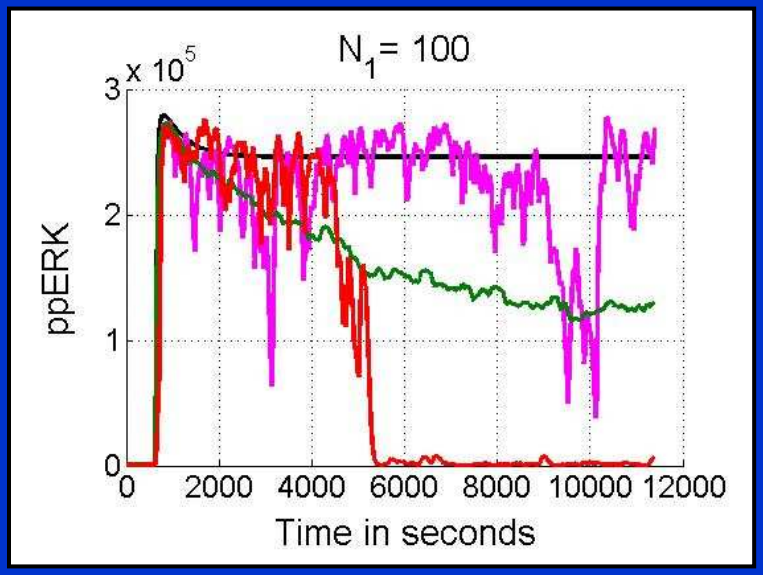
Monostable





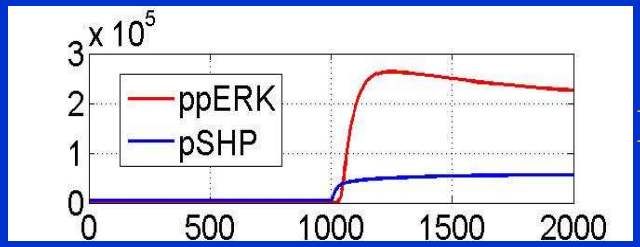
Bistable



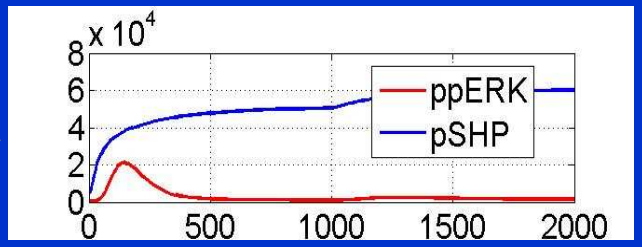


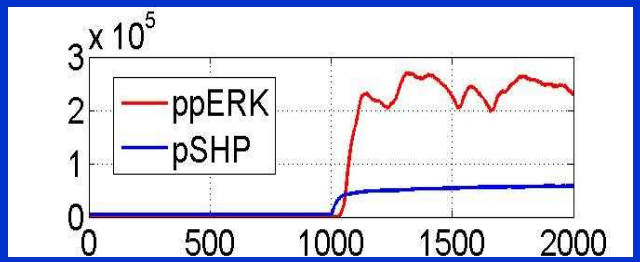
Primed

Inhibited

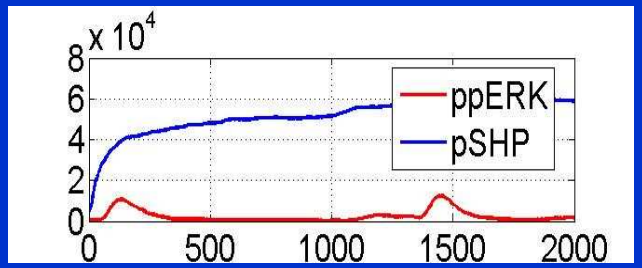


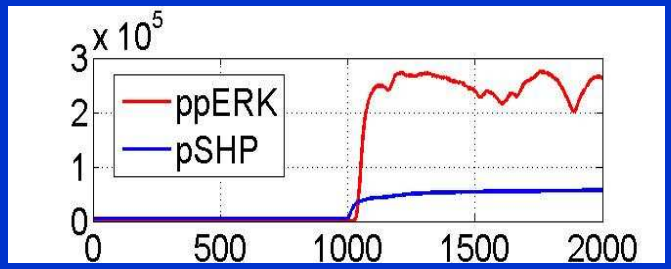
Deterministic



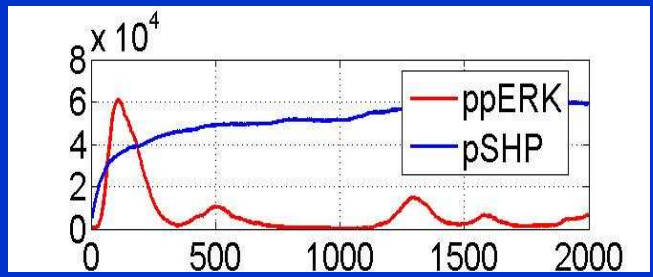


Stochastic





Stochastic



Time in seconds

Agonist and antagonist stimulation starts at t=1000s

Time in seconds

Antagonist stimulation starts at t=0, agonist stimulation starts at t=1000s

Conclusions

- Discrimination between agonist, endogenous and antagonist peptides is due to kinetic proofreading and competition of positive and negative feedbacks

- The system exhibit bistability and high stochasticity

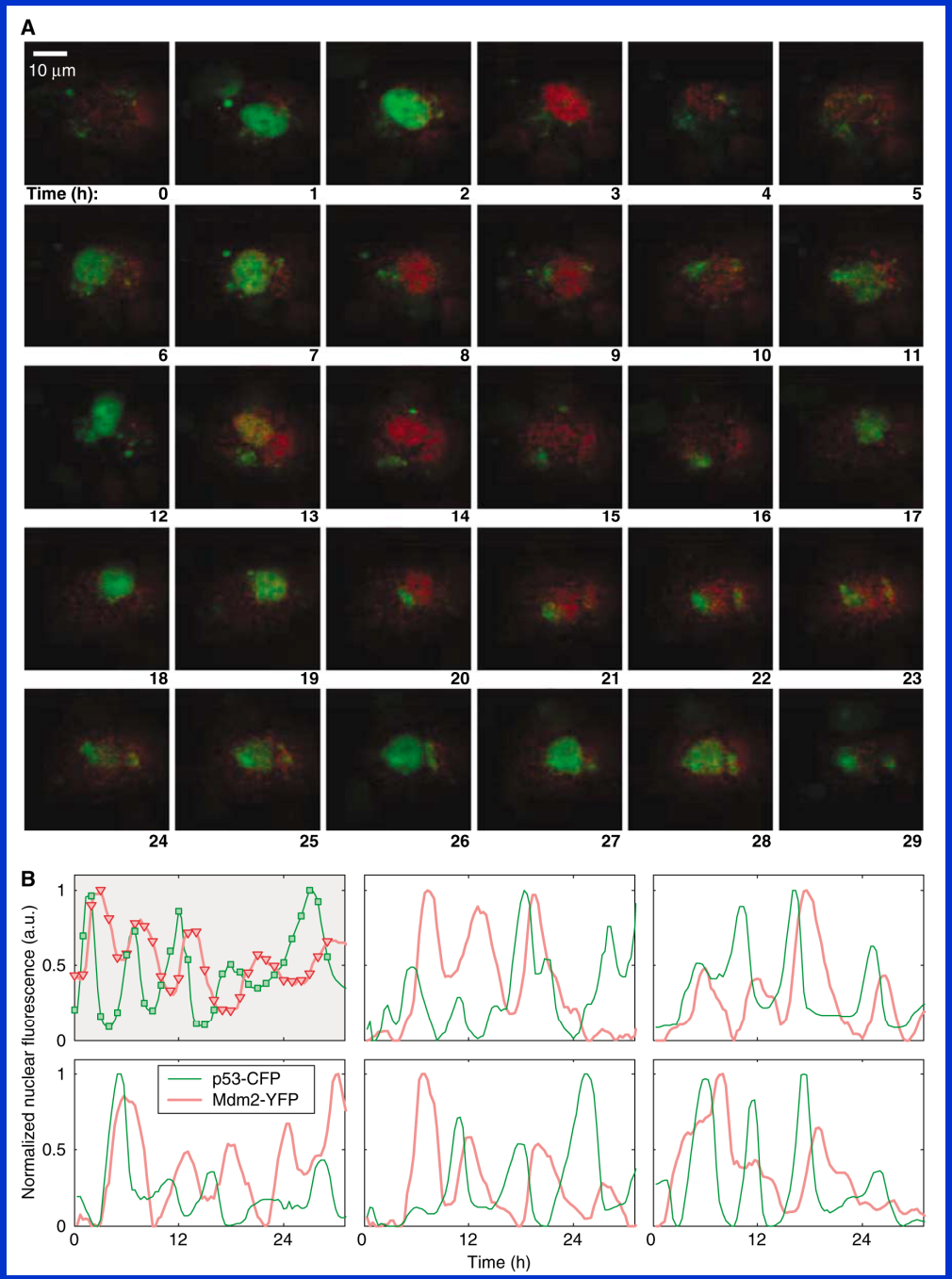
- This lead to a specific competition: bistability eases cell fate decisions while stochasticity makes that these decisions are reversible

Stochastic model of p53 regulation

Krzysztof Puszynski, Beata Hat, Tomasz Lipniacki

Why p53?

- p53 is a transcription factor that regulates hundreds of resposible for
  - DNA repair,
  - cell cycle arrest
  - apoptosis (programmed cell death)
- p53 is mutated (or absent) in 50% of solid tumors,
  in other 50% gene controlling p53 are mutated.
- 50 000 experimental citations, less than 100 theoretical papers



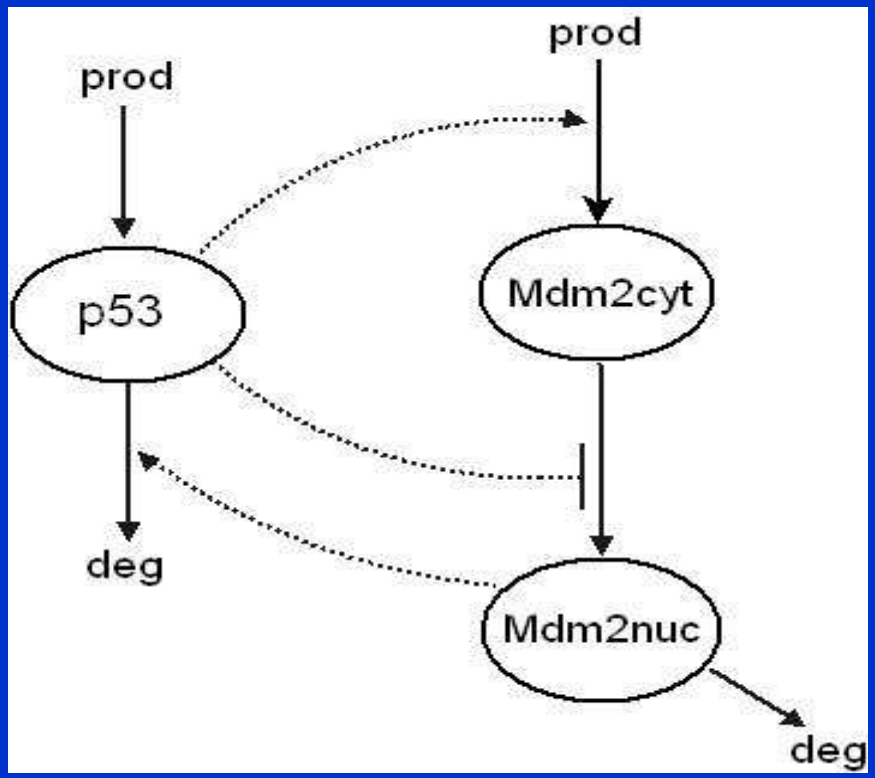
Single cell experiment (Geva-Zatorski et al. 2006)

- continuous oscillations for 72 hour after gamma irradiation

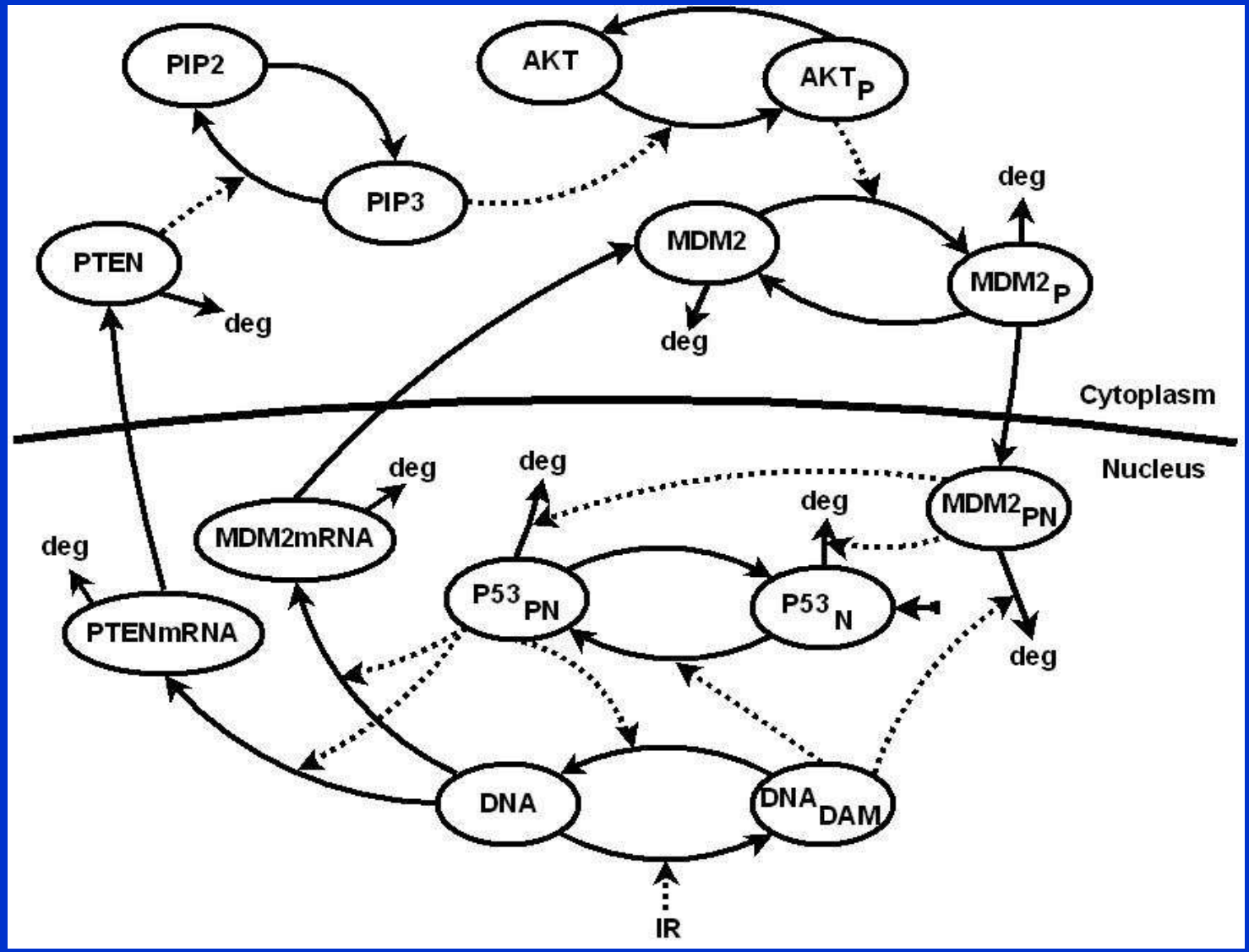
- fraction of oscillating cells increases with gamma dose reaching about 60% for 10 Gy.

- even after 10 Gy dose, analyzed cells proliferated

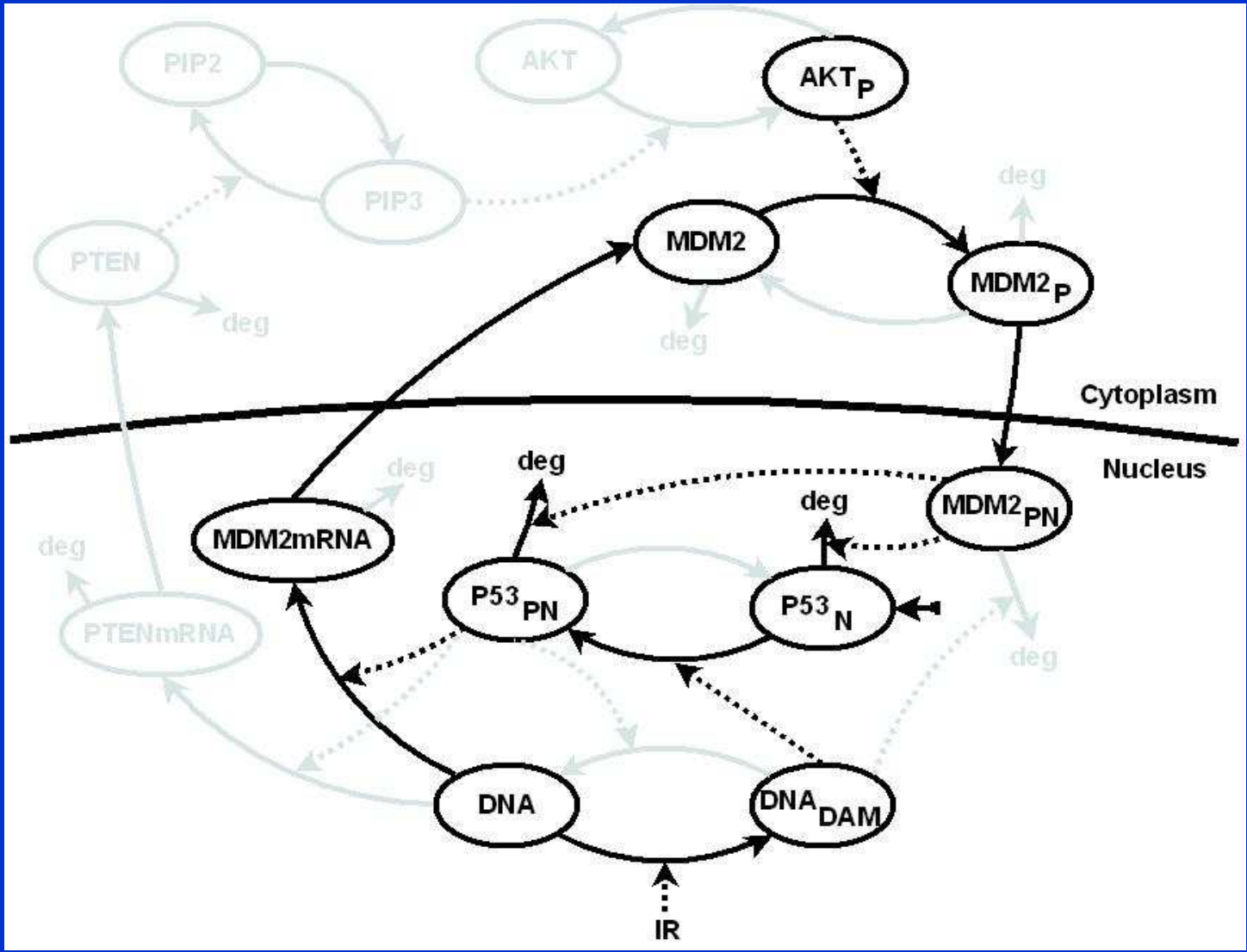
Negative feedback +
Positive feedback with time delay



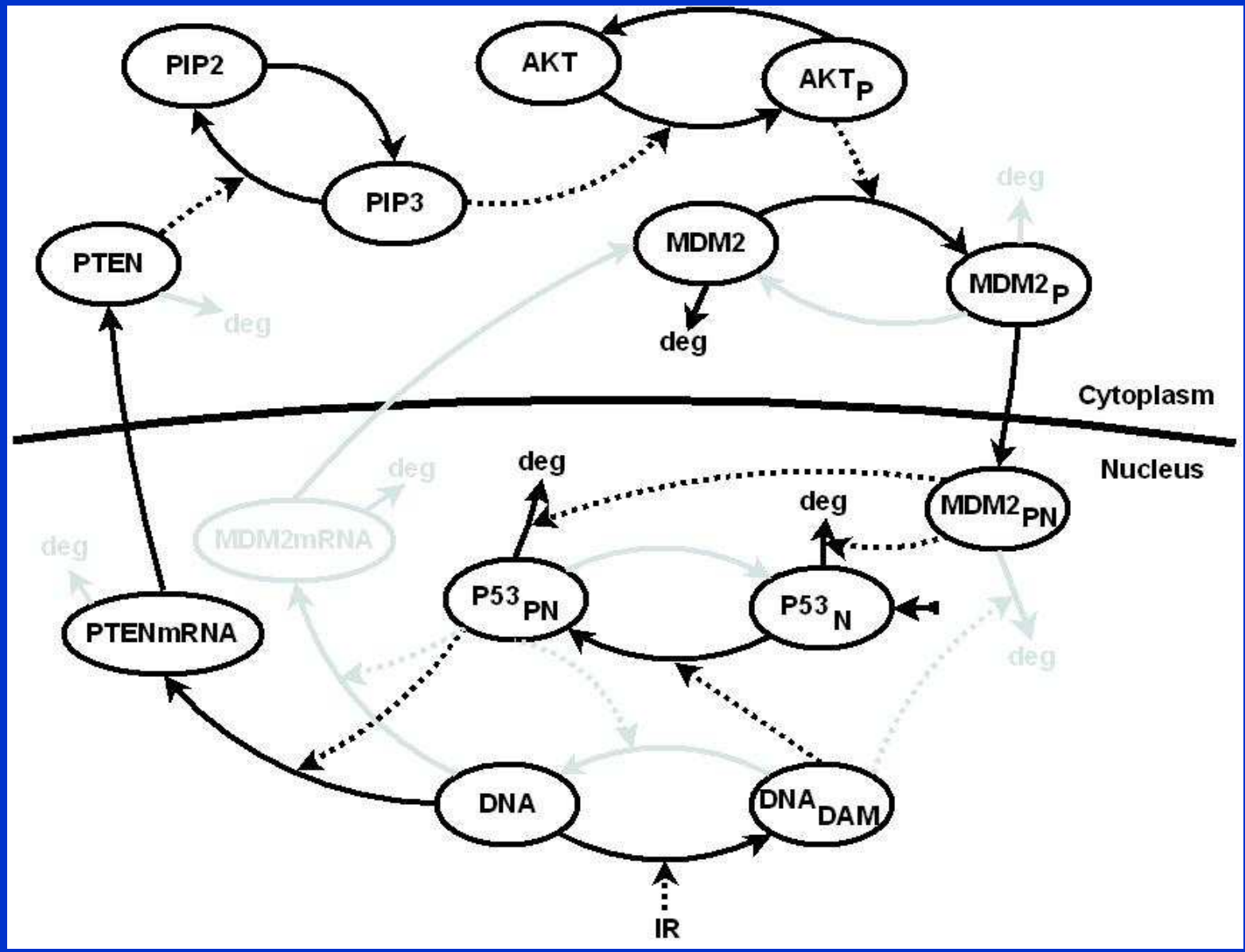
“Our pathway”


PIP2
AKT
AKT P
PIP3
deg
PTEN
MDM2
MDM2 P
deg
deg
Cytoplasm
Nucleus
deg
deg
deg
MDM2PN
MDM2mRNA
deg
P53 PN
P53 N
PTENmRNA
deg
deg
DNA
DNA DAM
IR

Negative feedback loop

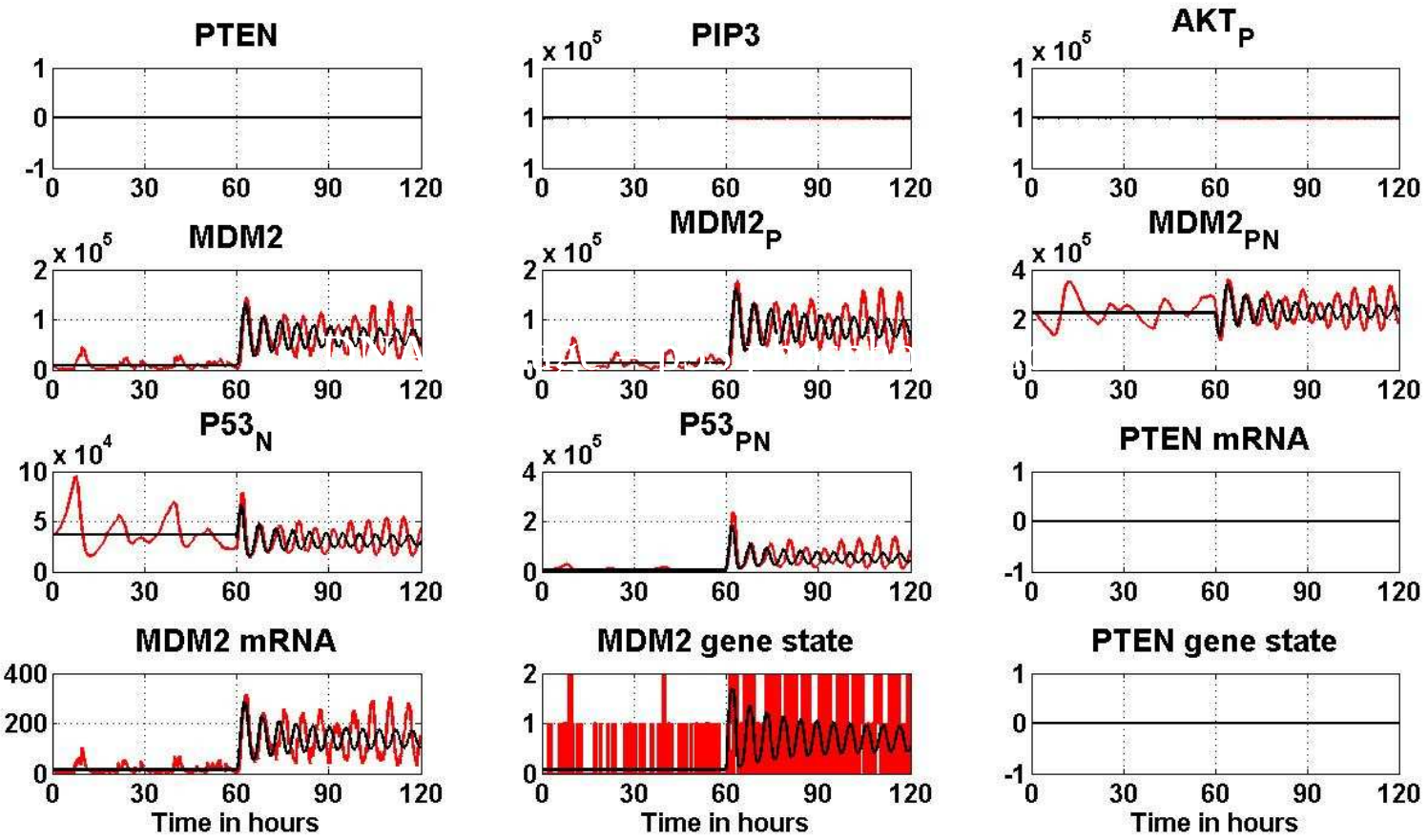


Positive feedback loop



No PTEN (positive feedback blocked); No DNA repair

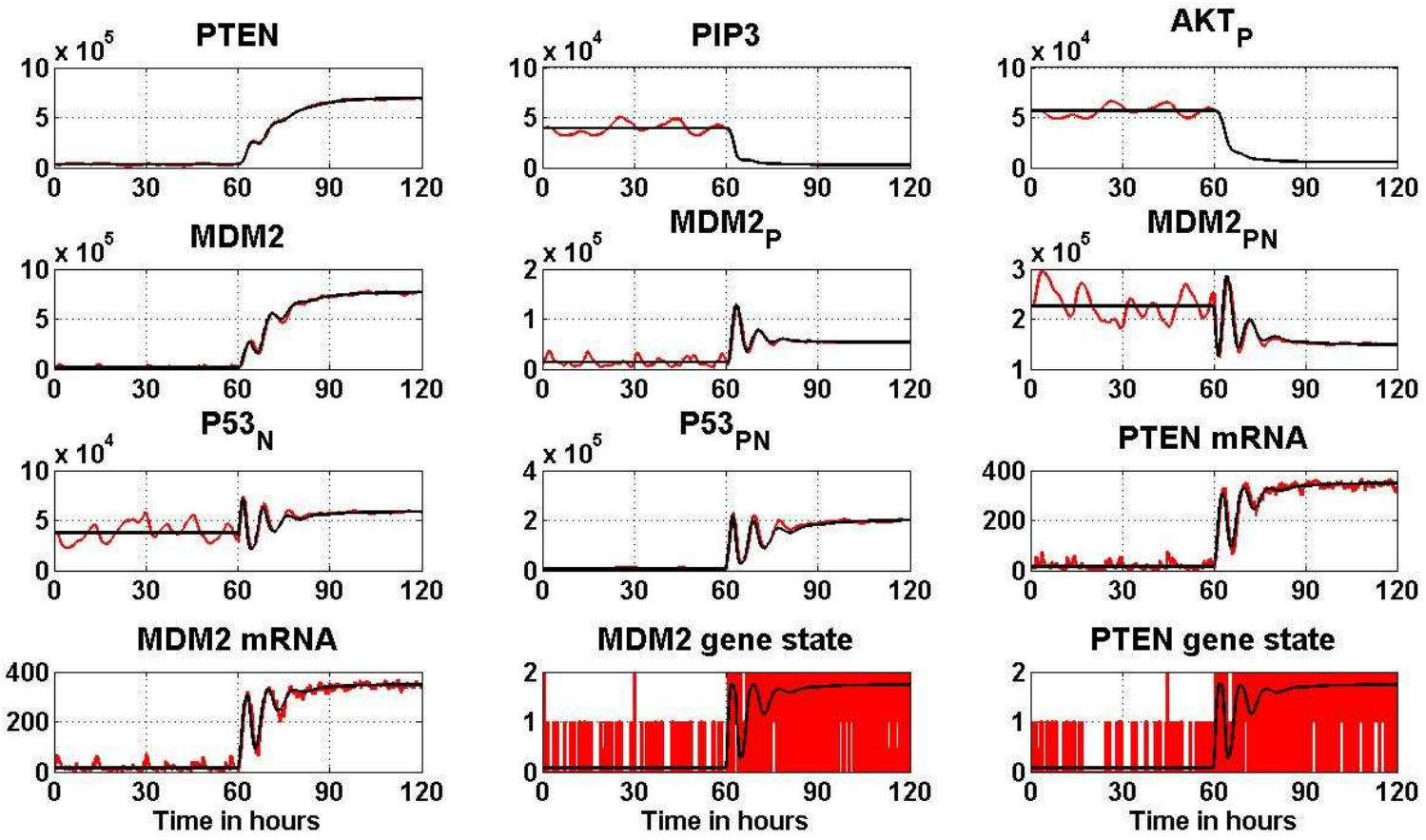
Oscillations



DNA damage = p53 phosphorylation + MDM2 degradation

PTEN *ON* (positive feedback active); No DNA repair

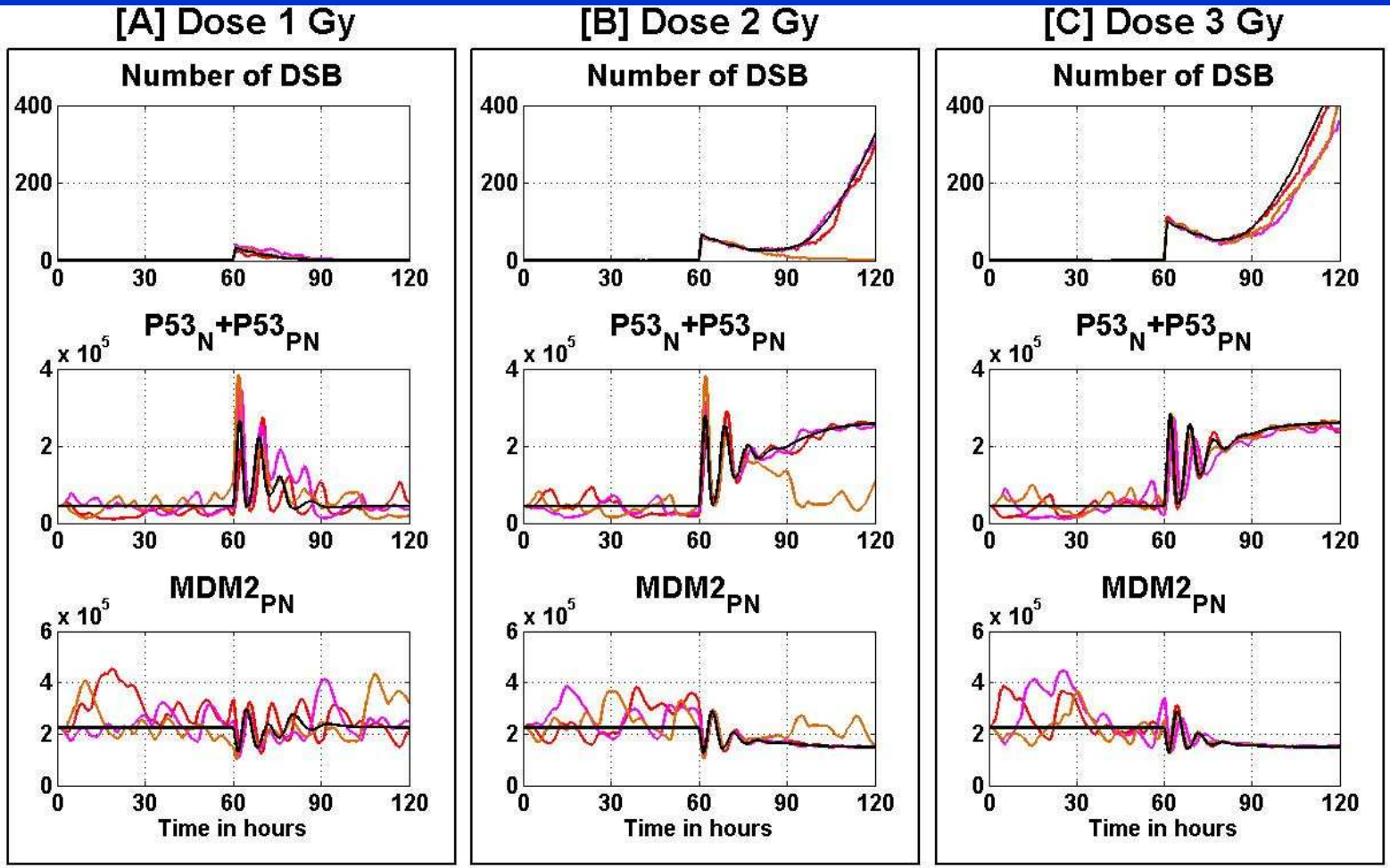
Apoptosis



DNA damage = p53 phosphorylation + MDM2 degradation

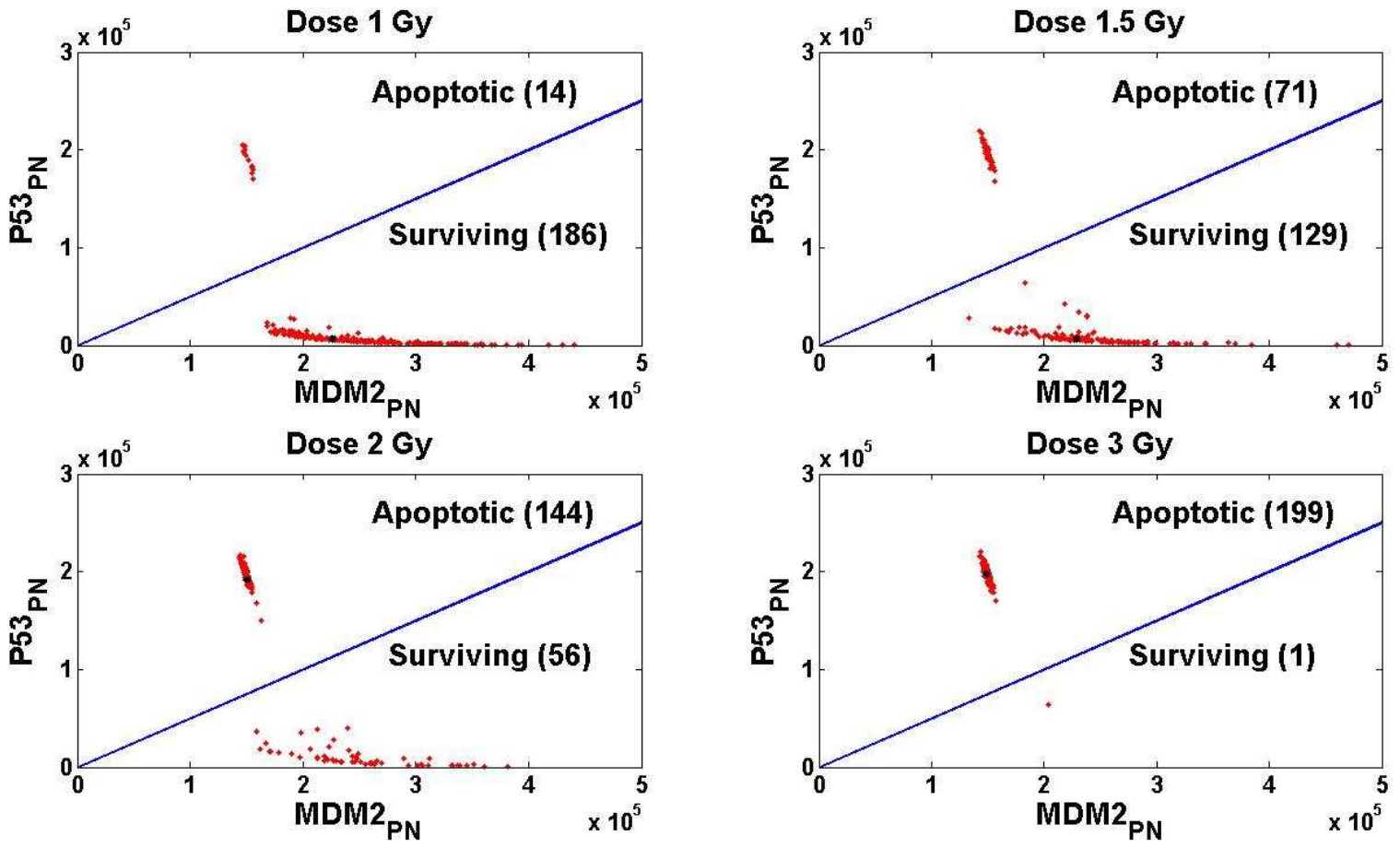
PTEN *ON* (positive feedback active); DNA repair *ON*

cell fate decision



p53 produces proapoptotic factor, which cuts DNA

Cell population separates into surviving and apoptotic cells 48 hours after gamma radiation.



ODEs

$$\frac{d}{dt}PTEN(t) = t_1\ PTEN_t(t) - d_2\ PTEN(t).$$

$$\frac{d}{dt}PIP_p(t) = a_2\ \left(PIP_{tot} - PIP_p(t)\right)\ - c_0\ PTEN(t)\ PIP_p(t).$$

$$\frac{d}{dt}AKT_p(t) = a_3\ \left(AKT_{tot} - AKT_p(t)\right)\ PIP_p(t) - c_1\ AKT_p(t).$$

$$\begin{aligned}\frac{d}{dt}MDM(t) \quad &= \quad t_0\ MDM_t(t) + c_2\ MDM_p(t) \\ &\quad -a_4\ MDM(t)\ AKT_p(t) - \left(d_0 + d_1\ \frac{N^2(t)}{h_0^2 + N^2(t)}\right)\ MDM(t).\end{aligned}$$

$$\begin{aligned}\frac{d}{dt}MDM_p(t) &= a_4\, MDM(t)\, AKT_p(t) - c_2\, MDM_p(t) - i_0\, MDM_p(t) \\ &\quad + e_0\, MDM_{pn}(t) - \left(d_0 + d_1\, \frac{N^2(t)}{h_0^2 + N^2(t)}\right)\, MDM_p(t).\end{aligned}$$

$$\frac{d}{dt}MDM_{pn}(t) = i_0\, MDM_p(t) - e_0\, MDM_{pn}(t) - \left(d_0 + d_1\, \frac{N^2(t)}{h_0^2 + N^2(t)}\right)\, MDM_{pn}(t).$$

$$\begin{aligned}\frac{d}{dt}P53_n(t) &= p_0 - \left(a_0 + a_1\, \frac{N^2(t)}{h_0^2 + N^2(t)}\right)\, P53_n(t) + c_3\, P53_{pn}(t) \\ &\quad - \left(d_3 + d_4\, MDM_{pn}^2(t)\right)\, P53_n(t).\end{aligned}$$

$$\begin{aligned}\frac{d}{dt}P53_{pn}(t) &= \left(a_0 + a_1\, \frac{N^2(t)}{h_0^2 + N^2(t)}\right)\, P53_n(t) - c_3\, P53_{pn}(t) \\ &\quad - \left(d_5 + d_6\, MDM_{pn}^2(t)\right)\, P53_{pn}(t).\end{aligned}$$

$$\frac{d}{dt}MDM_t(t) = s_0\,(G_{M1} + G_{M2}) - d_7\,MDM_t(t).$$

$$\frac{d}{dt}PTEN_t(t) = s_1\,(G_{P1} + G_{P2}) - d_8\,PTEN_t(t).$$

proapoptotic factor

$$\frac{d}{dt}A(t) = p_1\frac{q_3\,P53^2_{np}(t)}{q_4 + q_3\,P53^2_{np}(t)} - d_9 A(t)$$

Transition probabilities governing dynamics of discrete variables; G_M, G_P, N

Gene activation: $P^b(t, \Delta t) = \Delta t \times (q_0 + q_1 \times P53^2_{np}(t)).$

Gene inactivation: $P^d(t, \Delta t) = \Delta t \times q_2.$

DNA damage: $P^{DAM}(t, \Delta t) = \Delta t \times d_{DAM} \times R + \Delta t \times a_6 \left(\frac{A(t)}{A_{\max}}\right)^4$

DNA repair: $P^{REP}(t, \Delta t) = N(t) \frac{\Delta t \times d_{REP} \times P_A(t)}{N(t) + N_{SAT} \times P_A(t)}$

Piece-wise deterministic, time continuous Markov process

Numerical implementation

1. At the simulation time t for given A_{Mdm2}, A_{PTEN} and NB calculate total propensity function of occurence of any of the reaction

$$r(t) = r^a_{DNA} + r^d_{DNA} + r^a_{Mdm2} + r^d_{Mdm2} + r^a_{PTEN} + r^d_{PTEN}$$

2. Select two random numbers p_1 and p_2 from the uniform distribution on (0,1)
3. Evaluate the ODE system until time $t+\tau$ such that:

$$\log(p_1) + \int_t^{t+\tau} r(s)ds = 0$$

4. Determine which reaction occurs in time $t+\tau$ using the inequality:

$$\sum_{i=1}^{k-1} r_i(t+\tau) < p_2 * r(t+\tau) \le \sum_{i=1}^{k} r_i(t+\tau)$$

where k is the index of the reaction to occur and $r_i(t+\tau)$ individual reaction propensities

5. Replace time $t+\tau$ by t and go back to item 1

Stochastic robustness of NF- κB signaling

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With thanks to

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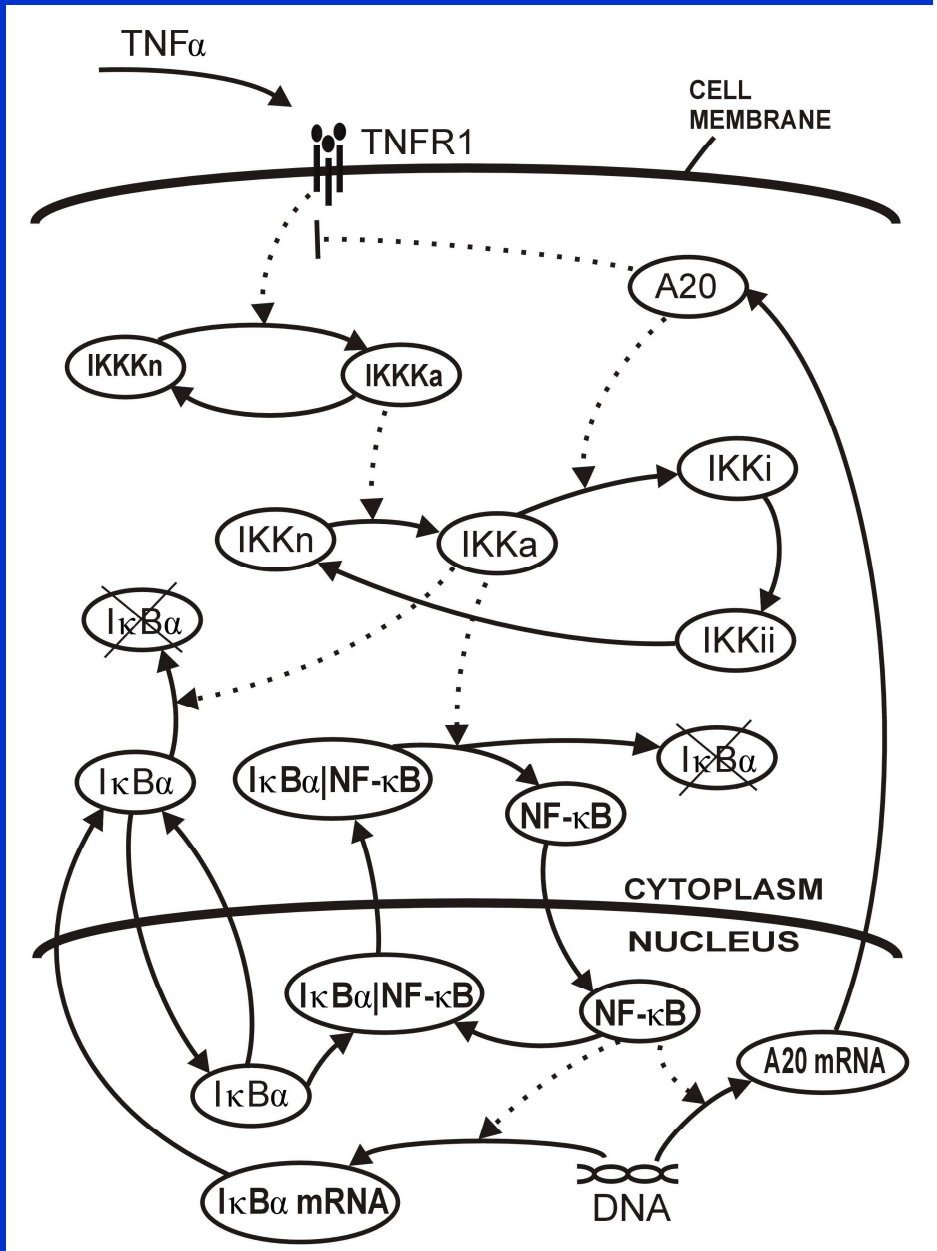
Two feedback model of NF-κB dynamics

- Key players:
  - NF-κB (transcription factor)
  - IκBα (inhibits NF-κB)
  - IKK (destroys IκBα)
  - IKKK (activates IKK)
  - TNFR1 (activates IKKK)
  - A20 (inactivates IKK)

- Feedbacks
  - NF-κB promotes transcription of IκBα
  - NF-κB promotes transcription of A20

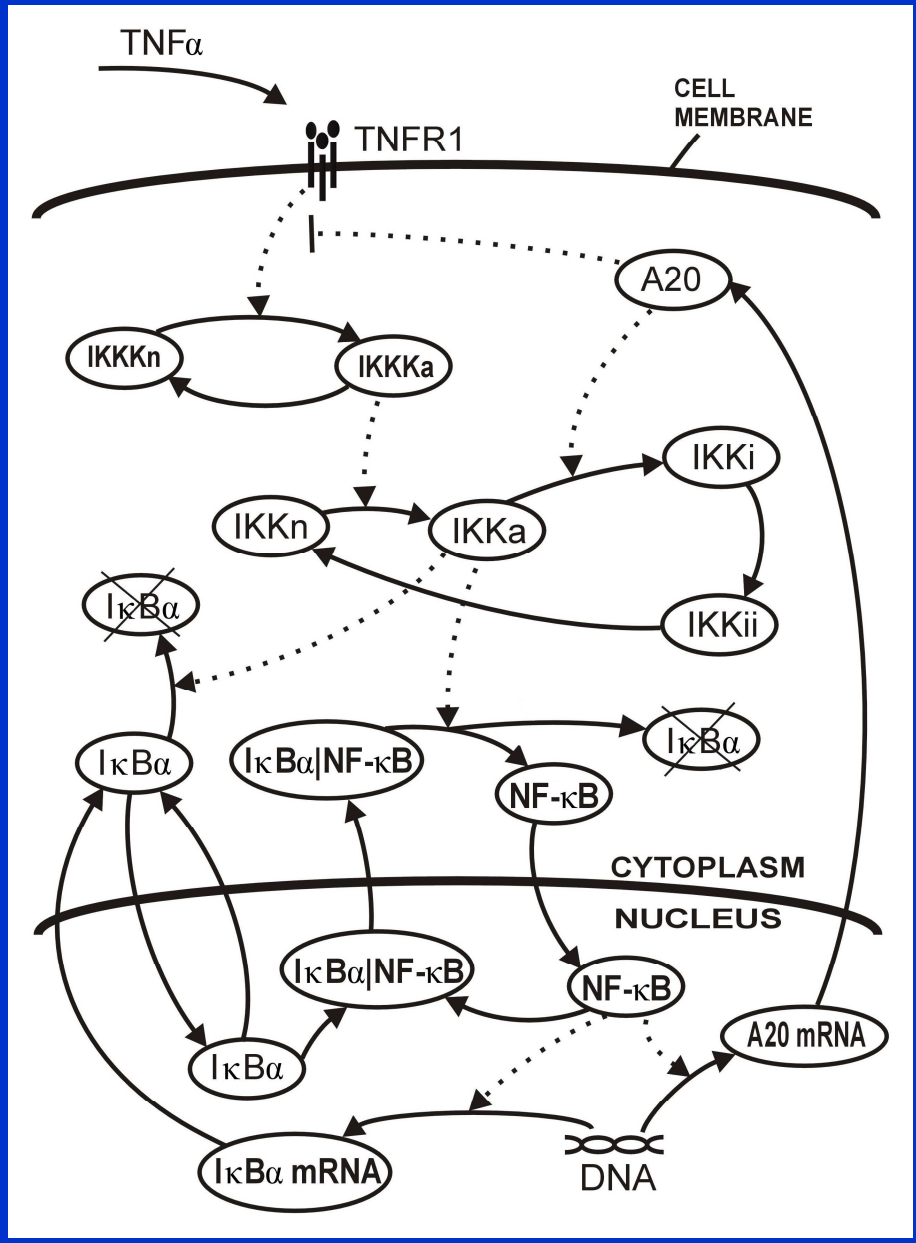


The model: processes considered

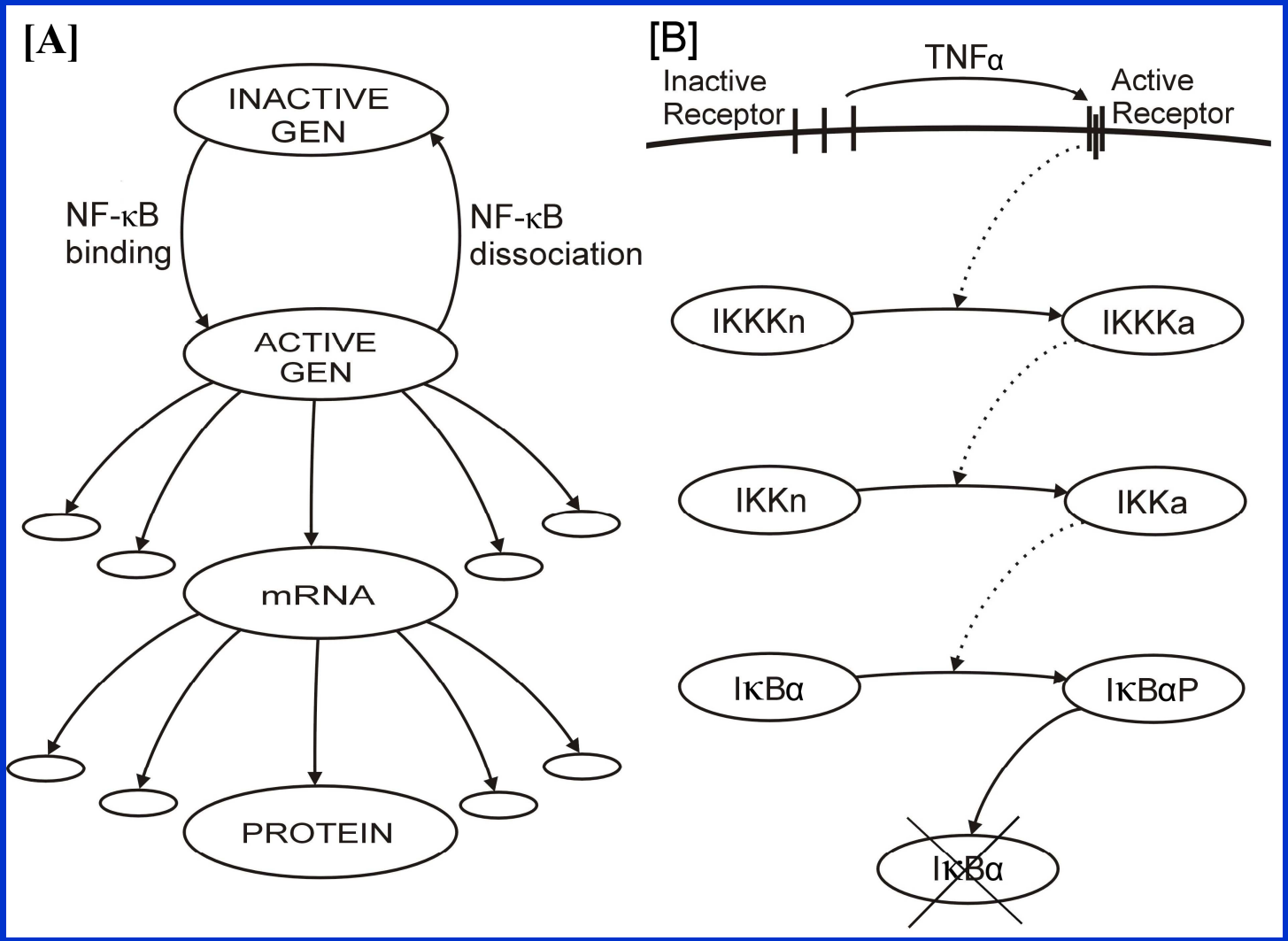
- Stochastic: receptors and genes activation
- IKKK activation
- IKK activation, IKKa->IKKi
- Synthesis of protein complexes
- Catalytic degradation of IκBα
- mRNA transcription
- mRNA translation
- Transport between compartments

Modeling: 15 ODEs

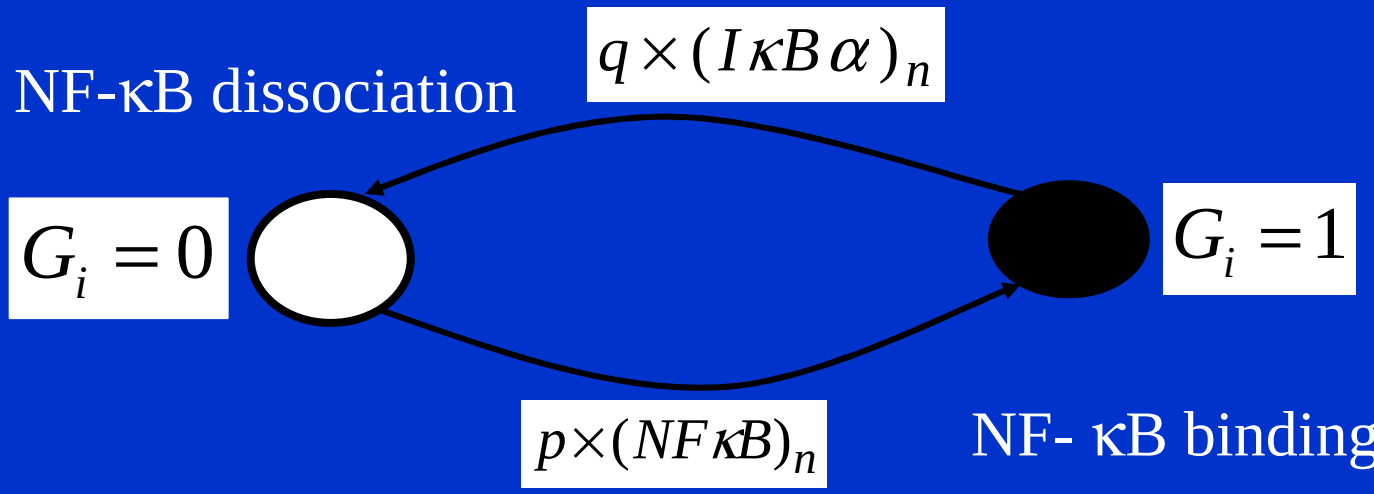
+ Stochastic switches for gene and receptors activities.



Stochastic switches and amplification cascades



Stochastic gene activation

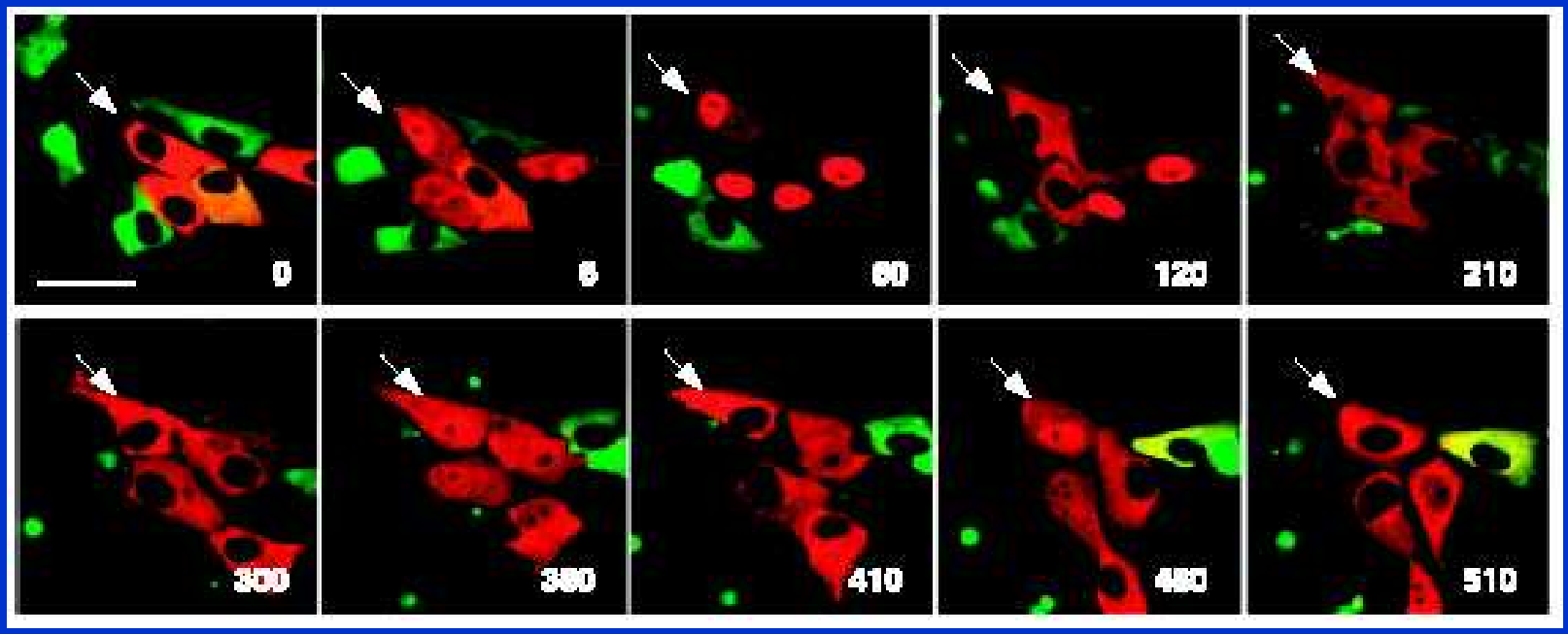


Gene activity G is a sum of activities G_i of *n* homologous gene copies.

$$G = \sum_{i=1}^{n} G_i$$

Nelson et al, Science 2004 (M.R.H. White group)

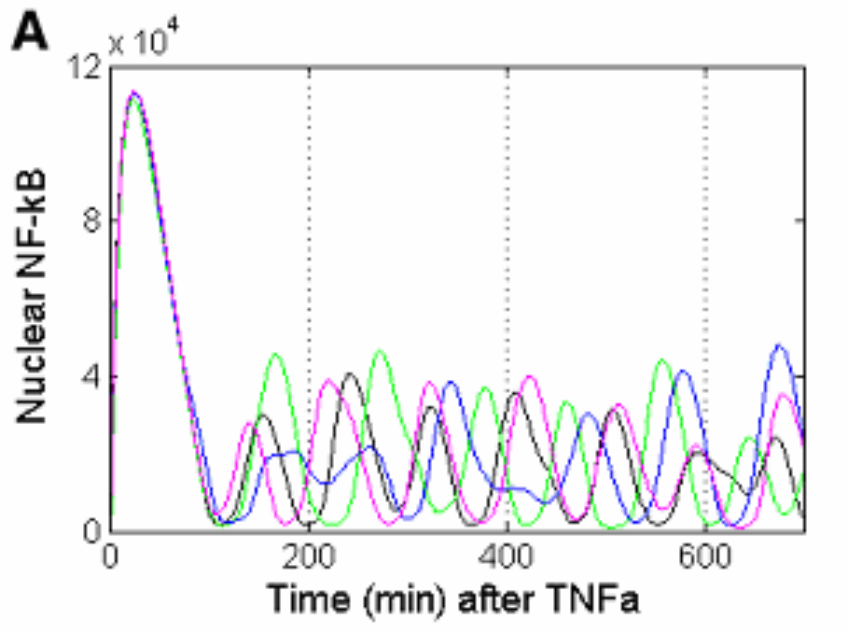
Tonic TNF stimulation

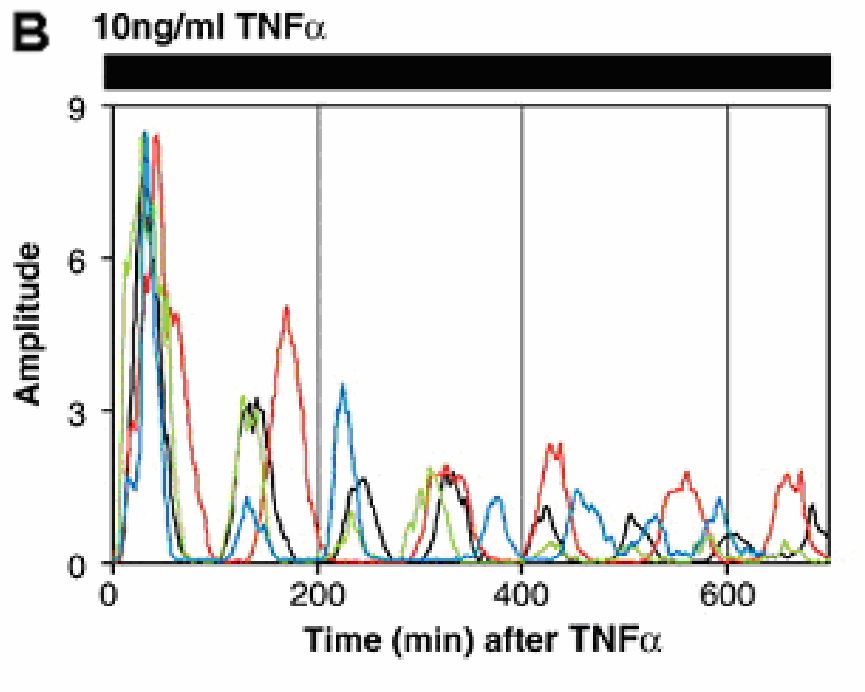


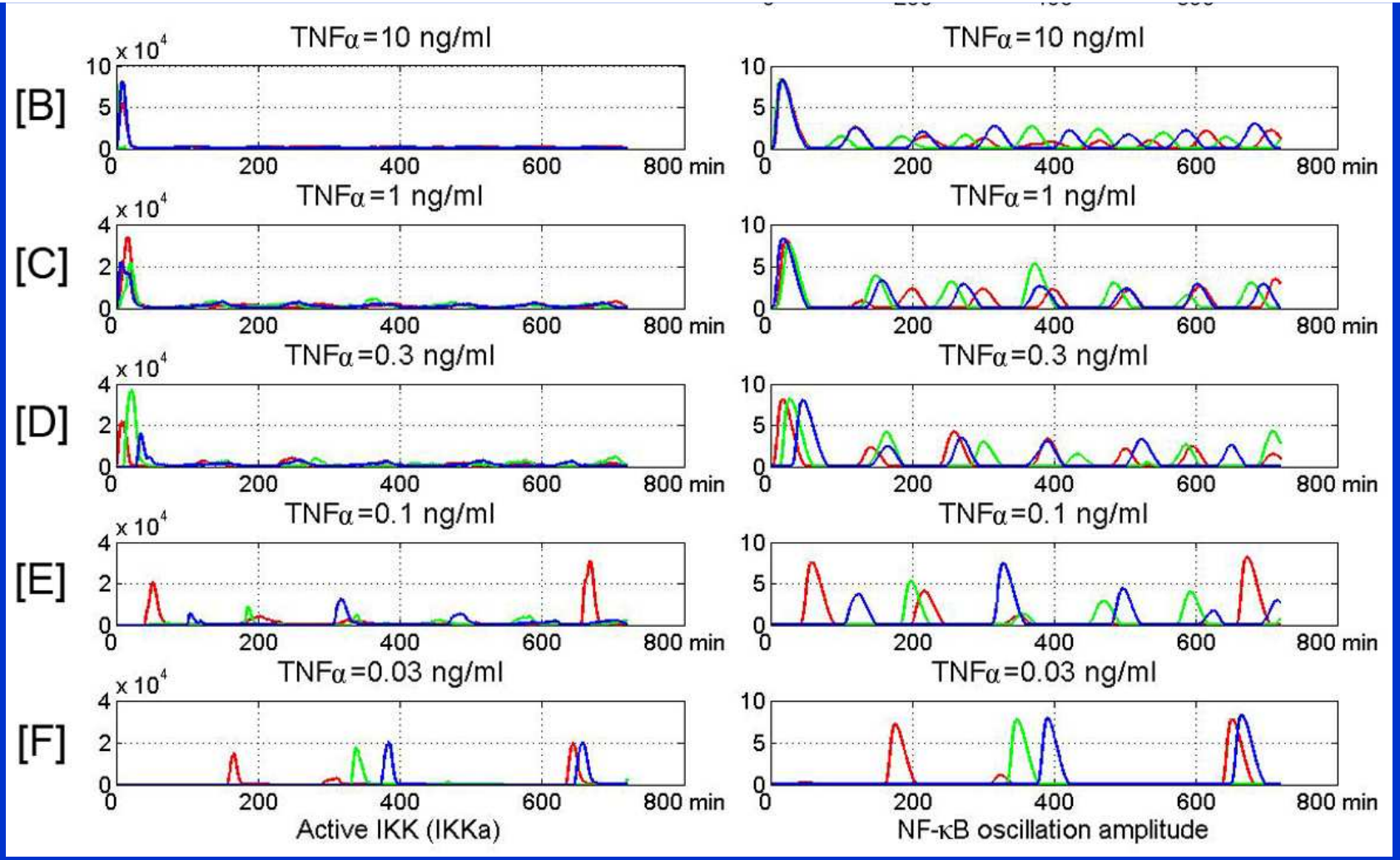
SK-N-AS (human S-type neuroblastoma cells) expressing
RelA-DsRed (RelA fused at C-terminus to red fluorescent protein)
and
IkBa-EGFP (IkBa fused to the green fluorescent protein)

Comparing model predictions with single cell experiment,

Nelson et al, Science 2004 (M.R.H. White group)

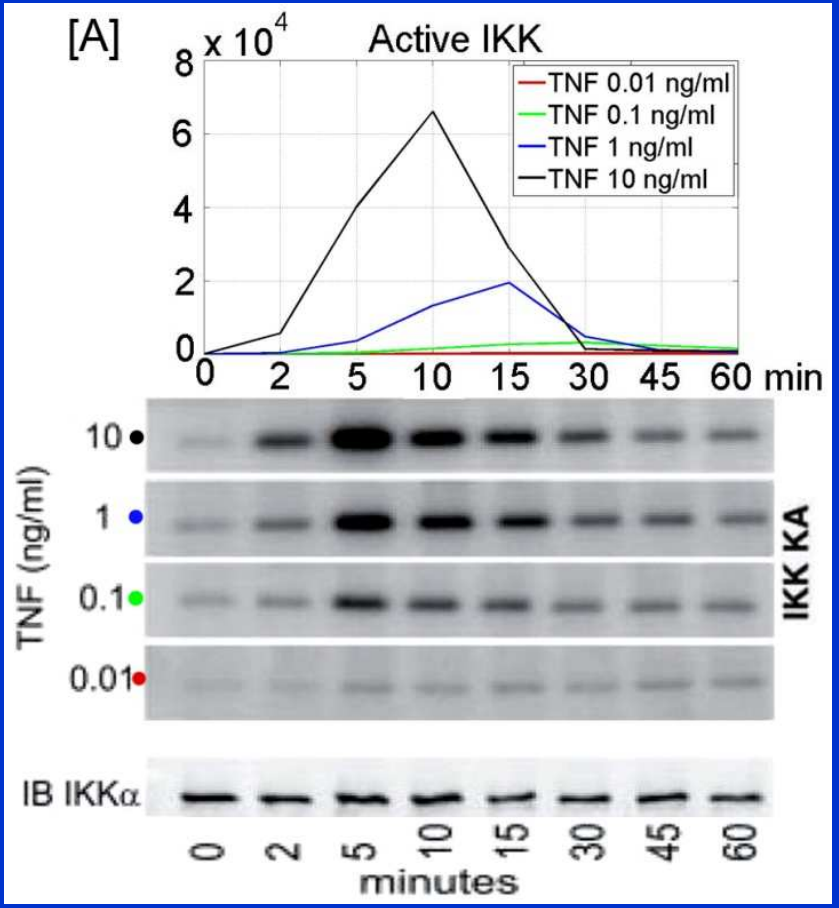


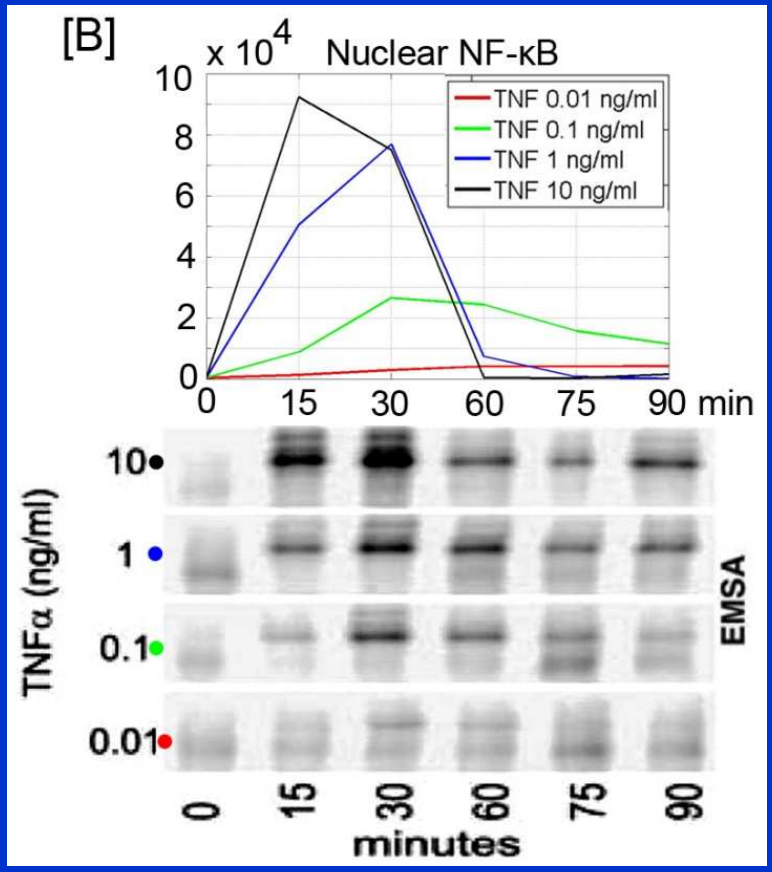




Single cell simulations for various TNF doses

Small TNF dose





Cheong R et al. (2006) J Biol Chem 281: 2945-2950.

Conclusions

Stochasticity as a way of defense:

High dose:

First 1.5h: same for all cells (inflamatory genes), then different (late genes activation)

Low dose:

some cells respond some not, the minimum response is quite strong.